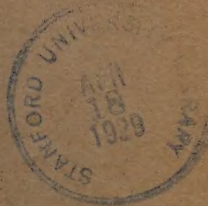


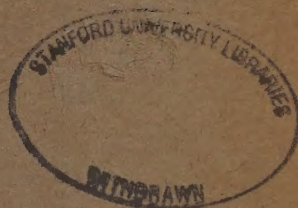
GERMINATIVE ENERGY OF LOTS OF CONIF-
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CUBATION TEMPERATURE AND TO
DURATION OF INCUBATION

BY
FERDINAND W. HAASIS



Dissertation submitted to the Board of University
Studies of the Johns Hopkins University in
conformity with the requirements
for the degree of Doctor
of Philosophy

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[Reprinted from PLANT PHYSIOLOGY, 3: 367-412, 1928]

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GERMINATIVE ENERGY OF LOTS OF CONIFEROUS-TREE SEED,
AS RELATED TO INCUBATION TEMPERATURE
AND TO DURATION OF INCUBATION¹

FERDINAND W. HAASIS

(WITH FIFTEEN FIGURES)

Introduction

The rate at which heat passes into or out of the plant body varies continually and the temperature of the plant always tends to approach that of the surroundings, from which it usually differs but slightly. The temperature of the surroundings may consequently be considered as an index of the concurrent plant temperature, and environmental temperature (as of soil and air) is usually looked upon as one of the chief influences that determine physiological activity. It is becoming increasingly appreciated that the influences exerted by temperature upon organisms are themselves dependent, however, on concurrent influences of other kinds. Some of these non-temperature influences are primarily related to, or bound up in, the internal characteristics of the organism or tissue considered, while others are active more or less directly at the external periphery of the organism and consequently operate inward from the environment. Whether any individual seed may germinate at all, for example, or how rapidly the physiological processes of germination may occur, with a specified set of temperature conditions, clearly depends only partly on the temperature complex itself, for the kind of seed considered, its physiological condition, etc., must obviously be important among the controlling conditions. The germination response of any viable seed to a given temperature complex is dependent upon many environmental conditions other than those of temperature; with a temperature complex that would otherwise give rapid

¹ Botanical contribution from the Johns Hopkins University, no. 104.

germination, this process may be greatly retarded or even wholly inhibited if the rate of water supply or of water loss, the rate of oxygen supply, or the rate of supply (or of removal from the surrounding region) of poisonous or stimulating materials (such as salts and organic compounds) is either too high or too low for satisfactory development of the organism when under the influence of the particular temperature complex in question. This recently familiar line of thought leads inevitably to the proposition that no statement about the temperature relations of vital processes can be more than very superficially useful, in regard to the definite analysis of physiological control and causation, unless the concomitantly effective, non-temperature conditions as well as those of temperature, are adequately defined. Of course the definition or description of these background influences cannot as yet be very precise in many respects, but they need to be and they can be clearly enough set forth to make it possible for other experimenters to approach them to an adequate degree.

Any given environmental temperature complex has several characteristics. The intensity or degree of the temperature obtaining in or around the organism considered (conveniently expressed as degrees on a thermometer scale) is first thought of; we may say that a seed germinated at a temperature of 25° C. But the temperature of a germinating seed is seldom even approximately maintained for any considerable period of time and temperature fluctuation consequently requires special consideration. Temperature may fluctuate very little (being nearly maintained) or it may fluctuate greatly and the fluctuations, whether small or large, may be slow or rapid in regard to time, often differing in rate for different portions of the experiment period. As has been emphasized by MACDOUGAL (14), if the temperature is not practically maintained the form of the thermographic curve or tracing for any time period may be fully as important physiologically as are the corresponding minimum and maximum temperature values. Finally, whatever temperature complex is dealt with, whether the temperature of the organism is nearly maintained or fluctuates according to some adequately described pattern, the duration factor is of course of prime importance. Any statement of a physiological temperature relation must be considered as holding only for the exposure period for which that statement is made. We may say that a certain seed germinated with a maintained temperature of 25° (with regular or irregular fluctuations of plus or minus 1° , for example), but our statement is without much value unless we add that germination occurred within a certain number of hours, days, etc., following the beginning of incubation. Some of these considerations of the nature of temperature influence on organisms have received attention from FAWCETT (6).

Technique and rational analysis have not yet progressed sufficiently to encourage the undertaking of serious experimental studies on the influence exerted on physiological processes by different forms of temperature fluctuation, and most of the investigations thus far reported in physiological literature have dealt with approximately maintained temperatures. From the point of view of ecological science (including forestry, agriculture, hygiene, etc.), for which natural conditions must be studied, temperature change has received some attention, but before the influence of fluctuating temperature can be approached by analytically planned experimentation it will be necessary that our knowledge of the much simpler maintained-temperature relations of organisms and tissues be greatly increased.

Many aspects of plant temperature relations are of great importance in plant ecology and the applied sciences that rest on plant physiology. This is especially true in regard to seed germination, for the germinating seed is usually so intimately and simply related to its surroundings that its temperature relations offer excellent opportunities for experimental study and for the application of physiological principles. Also from the standpoint of physiology itself seed germination is of exceptional interest, for it presents fewer difficulties than most of the other phases of plant activity and the complexity of its relations is correspondingly less discouraging to the experimenter. Furthermore, seed germination forms the starting-point for many different kinds of experiments upon the conditions that influence other plant processes, and precise knowledge and exact appreciation of the causal controls of germination are required for the working out of standard treatments calculated to produce a number of physiologically similar plants for experimental study. Experimentation on any aspect of plant activity is not apt to advance either rapidly or far until it is possible to secure at will lots of plants that are nearly enough alike for the special requirements of the investigation in hand. It may be added that the germinating seed furnishes us with a very compact and relatively simple vital system, which embraces most of the essential physiological processes. Seed germination and the growth of young seedlings have always been a favorite field for experimentation of both the cruder and the more refined types.

Comparatively few studies have been made on the temperature relations of seed germination from the standpoint of the fundamental analysis of determining conditions or controls, and the work here reported was planned to throw a little additional light into this particular corner of plant physiology and to furnish some aids and sign-posts that may be more or less useful to future workers who may wish to explore this field more thoroughly. From the nature of the problems involved, this study is of course of only

preliminary and exploratory nature. The concrete or immediate object was the determination of the influence of maintained temperature, acting for various lengths of time, upon the germinative energy of lots of seed of several different species of coniferous trees, especially pitch pine (*Pinus rigida* Mill.), the percentage of germination occurring under the test conditions being considered as the index of germinative energy.* A fairly extensive study of rice seed (*Oryza sativa* L.) was also made and seed of other agricultural plants was studied to some extent, but only the results obtained with seed of certain pines and other conifers will be presented in this publication.

It was especially desired to find out the optimal temperature for the occurrence of a high percentage of germination under the general conditions of the experimental tests and with different lengths of the incubation period, but attention was also paid to minimal and maximal temperatures and to the forms or patterns of the temperature-germination graphs. An attempt was made to employ more or less standard treatments of the seed samples with regard to the many influential conditions aside from temperature and the duration of the incubation period, but the aim of the study was not to find out just how to specify an environmental complex that would produce high percentages of germination in various lots of seed. That would constitute a practical problem that might promise the possibility of more immediate application in forestry and agriculture, but it might prove to be too complex for attack until a number of much more restricted and scientifically fundamental questions had been already answered much more clearly than is now possible. Indeed, the present study may be considered as an attack on one, or a very few, of the great array of such fundamental questions that must occur repeatedly to every student of physiology and the related applied sciences. It is hoped that the non-temperature conditions of the environment that were effective in these experiments are clearly enough described, in terms of the technique by which they were secured, so that other experimenters may be able to approach them to a fairly satisfactory degree. For some maintained temperatures and for some lengths of incubation period, the technique used surely furnished very satisfactory environmental complexes for the germination of large proportions of the main lots of seed studied.

Several matters of definition and logical analysis deserve attention at the outset. Following the usage of seed testers, *germination percentage* or the *percentage of germination* denotes the average number of seeds that germinated per hundred seeds used in any test. A sample of seeds is ex-

* This use of the word *energy* may be confusing, since the word is not here employed in its physical sense, but "germinative energy" has been used in discussions of seed testing and is recommended by TOUMEY (22).

posed to the influence of an environment calculated to induce germination in some of them at least, the number found to have germinated at the end of a certain period of time is ascertained, and this number is expressed as a percentage of the number of seeds in the sample used. For any test, this numerical value is considered as an index of the germinative energy of the sample tested and, if the sample fairly represented the entire lot of seed from which it was taken, the germinative-energy index of the sample is taken as the germinative-energy index of the lot. Several samples of any lot of seed are generally subjected to like tests and the germinative-energy index of the lot is the average of the indices of the samples. On the basis of any particular kind of viability tests a given lot of seed is to be considered as made up of two categories of individuals, (A) those that are capable of germination and (B) those that are not. If, for example, ninety per cent. of the seeds in a lot are individually viable for the kind of test used we say that the viability of the lot is 90 per cent. on the basis of that kind of test, or that the percentage viability of the lot is 90, and that value is the index of the germinative energy of the lot for the particular environmental complex used in the tests from which it is derived.

The viability of a lot of seed thus exhibits a special kind of variability, with reference to the innate capacity of the lot to produce seedlings, and with reference to a specified set of environmental conditions. This concept is not to be confused with those of growth rate, respiration rate, etc., as such process rates are dealt with in physiological literature. The lot index of viability does not represent a rate, it represents merely the proportion of the seed population that are individually viable under the conditions of the test. Process rates, on the other hand, such as the rate of elongation of seedling roots, for instance, have not been generally studied with primary regard to the innate variability of the lots of seedlings or plants studied. Indeed, most experimenters with process rates subject their experimental material to somewhat careful selection before each series of tests is begun, the aim being to secure as high a degree of uniformity in seeds, seedlings, etc., as is possible. Thus, seeds are often selected for uniformity in size, color, weight and specific gravity, and seedlings are frequently selected for size, form, color, for previous rate of growth or other physiological capacity, etc. Such preliminary selection is essential in studies on the environmental control of physiological processes, as every one knows, for the results of such studies are valid only for the particular internal conditional complex that was represented by the lot of plants for which the results were derived. Because satisfactory uniformity in any lot of material is difficult to secure, it remains desirable, however, to give special attention to variability, even after uniformity has been approached as nearly as possible by various kinds

of preliminary selection. It is this thought that has led to the employment of elaborate statistical methods for the examination of experimental results that lend themselves to such treatment. Innate variability implies internal differences in physiological capacity, and hence in performance or behavior, among the several individuals of an experiment, and it constitutes at present perhaps the most serious obstacle to physiological and ecological progress. This statement applies especially to the logical analysis that is so important in the planning of experimental procedure as well as in the interpretation of experimental results.

Since innate variability is to be reduced as much as possible in experimentation on physiological control and since it is always present, in spite of everything an investigator may do, it becomes itself an important and very fundamental characteristic of any group of organisms, such as seeds or seedlings, cuttings or scions, etc. It requires attention and measurement at every turn. But variability is not a characteristic of the individual unit in an experimental series; it is of course a characteristic of the group only. In the sense here considered a single seed, for example, does not manifest variability. An individual seed possesses size, color, a certain amount of capacity for germination under any set of environmental conditions, etc., and the innate variability of a sample of seeds, however it may be measured, depends on the manner in which the several individuals of the sample differ from one another; an index of variability must characterize the physiological differences that obtain in the group. Knowledge of this group characteristic should permit selection for uniformity with regard to the several kinds of variability and should facilitate the interpretation of the numerical results of experiments on physiological processes. As has just been implied, such knowledge is also desirable whenever different lots of individuals are to be compared, as in ordinary seed testing, for example. The aim of the seed tester is not primarily to ascertain just how individual seeds differ, but how one lot of seed differs from another lot with respect to capacity to produce seedlings. Individual differences are studied primarily to obtain information by which the lot differences may be compared.

An index of germinative energy needs always to be stated with specific reference to the particular complex of environmental conditions with which the germinative-energy tests were made, for the index value cannot be considered as valid except for that complex. It is clear that the numbers of seeds germinating in two like samples and the germination percentages derived therefrom may be very different if the two samples are tested in different ways. Water conditions, oxygen conditions, temperature conditions, etc., are all influential, and especially the duration of the test period. For any lot of seed, for any set of non-temperature conditions and for a

stated length of the test period, the index of germinative-energy may have widely different values according to the temperature conditions of the test; for any lot of seed and for a stated set of physical and chemical conditions in the test, the value of the index of germinative energy may differ greatly according to the length of the period of incubation, etc. In short, the magnitude of the germinative-energy index is a function of a large number of variables, of which only a portion represent characteristics of the lot of seed considered, while the rest stand for the environmental conditions of the test and its duration.

Because of these considerations the germination percentage derived from any test of a sample of seeds is to be regarded only as corresponding definitely to the conditions and duration of the test. The index value may have any magnitude between zero and a maximum, the upper limit being determined by the characteristics of the lot of seed in question. It is consequently essential that we try to understand what are the environmental influences that affect the index value for any lot of seed and just how they operate to give that value any particular magnitude. Such an investigation as is here suggested may be approached only by means of analytically planned experimentation. For the present, most of the experimental conditions have to be relegated to the category of background conditions (to be described as adequately as possible and made uniform throughout the several tests of an experiment) while a very few may be considered, in any case, as experimental variables (to be studied with regard to differences in their intensities and qualities and always with special reference to the chosen background complex).

In the experiments dealt with in this paper all influences were in the background excepting (1) lot of seed, (2) maintained temperature and (3) duration of incubation. It will be seen in this report, for example, that the first lot of pitch pine seed tested gave, for the standard set of background conditions employed, many different indices of germinative energy, ranging from zero to about 90, the index values being environmentally influenced (always with specific reference to this particular set of background conditions) by temperature and duration of incubation. For shorter periods of incubation (not too short) the values are all low but they are higher with some maintained temperatures than with others. For longer periods of incubation they are very high with some maintained temperatures and very low with others. Whether the highest percentage of germination shown approaches the physiological and statistical limit for that lot of seeds remains questionable, but (since the highest value is close to 100 per cent.) it is clear that the maximum viability percentage was nearly attained.

An interesting feature of these results is the manner in which the form of the temperature-germination graph changes with the length of the incubation period, and it appears that the innate nature of any lot of seeds cannot be well understood unless this feature is experimentally studied. The optimal maintained temperature for the occurrence of germination in a given sample of seeds (or the temperature giving the highest index of germinative energy for that sample and for the test conditions) is not at all the same for short incubation periods as for longer ones, unless all periods are relatively long.

The individual seeds of any lot differ as to germinating power, and experimentation of the sort employed in these studies furnishes data for classifying the individual seeds in this respect. The results show what percentage of the lot (how many per hundred, on the average) may be expected to germinate under the influence of each set of test conditions (the duration factor being considered as one of the test conditions) and also what percentage of the lot may be expected to fail to germinate under the influence of each set. Failure to germinate does not necessarily imply lack of viability in a seed. Seeds that fail to germinate under one set of conditions may of course germinate under another set. For any given set of test conditions and for any incubation period, the seeds of any sample may thus be classified into germinable and non-germinable. For any set of non-temperature conditions and for any incubation period they may be classified further into several categories with regard to temperature. This question of the experimental classification of a lot of seeds is referred to in a subsequent section.

Different lots of seed are of course to be expected to differ in regard to statistical make-up, in spite of their being of the same species or variety, from the same individual plant, from the same inflorescence or even from the same capsule. Such differences between lots of seed, which are the differences with which a seed tester deals, are partly due to differences in the original proportions of "good" and "bad" seeds when the lots were harvested. Also, since stored seed is apt to deteriorate with time, the germinative-energy index of a given lot (for a particular kind of test) may alter more or less rapidly with time, according to the original make-up of the lot and the conditions of storage.

The analytical study of the temperature relations of vital phenomena has been approached by various methods and this whole field of science was reviewed, up to 1897 and 1914, respectively, by DAVENPORT (3) and KANITZ (8). A number of studies on the temperature relations of plants have dealt with the rate of elongation of roots and shoots of seedlings after germination has occurred. Examples are afforded by the studies of SACIS

(19), KOEPPEN (9), DE VRIES (4), LEHENBAUER (11), ISABELLA LEITCH (12) and NEWCOMBE (15). Since the studies considered in the present paper do not deal directly with rates of growth, they are not closely related to the contributions here cited and it is not necessary to present in this place a résumé of the latter.

Some investigators have studied the germinative energy of lots of seed as indicated by the percentage of germination when samples of each lot were exposed to the same or different environmental complexes for various incubation periods. Studies of this kind are somewhat more closely related to the present studies than are those dealing with rates of shoot or root enlargement, but the earlier results offer but little opportunity for analytical comparisons with those set forth in the present publication. Productive comparisons are generally precluded by the nature of the plans of experimentation that were followed. In this group may be mentioned reports by NOBBE (16), RAFN (18), DOROSHENKO (5), PACK (17), HARRINGTON (7), SIFTON (21), and especially those by BOERKER (2) and KORSTIAN (10).

NOBBE (16) aimed to find out what are the most suitable temperature conditions for germinative-energy tests of certain forest-tree seeds. He employed maintained temperatures and also studied the influence of alterations of maintained temperature. In the latter case a sample of seeds was exposed to the action of two different maintained temperatures, for different parts of the incubation period. His background conditions (considered as uniform for all cultures) were those of moisture and oxygen supply and his variables were temperature, length of incubation period and kind of seed. These studies were of course planned for practical application in seed testing rather than for the analysis of physiological control. The studies of HARRINGTON (7) were similar to those of NOBBE.

RAFN (18) aimed to have as background conditions all influences excepting kind of seed and duration of the incubation period. His studies included lots of seed of more than two hundred different tree species. DOROSHENKO (5), PACK (17) and SIFTON (21) employed as variables maintained temperature, duration of the incubation period and kind of seed.

BOERKER (2) studied the germinative energy of a number of kinds of tree seeds and considered as variables the kind of seed (species, race, size, etc.), the duration of the incubation period, the intensity of illumination (natural light modified by shade, in a greenhouse), soil-moisture content and soil texture. He gave little attention to temperature, however, and his air-temperatures, above the seed bed, fluctuated in irregular manner, between about 8° and about 38° C., when measured in the shade. The extent of the weekly temperature range was never less than 16° and it was

sometimes as great as 29° . His experiments lasted about six months and he regarded the temperature conditions as optimal throughout that period. The results are presented as graphs, in which ordinates represent percentage of germination and abscissas represent the length of the incubation period expressed in days.

KORSTIAN (10) studied the germinative energy of acorns of several species. His variables were the kind of seed (species), the duration of the incubation period (measured in five-day units) and the temperature conditions, other influences being about uniform for all tests and having values not far from what he regarded as optimal for the seeds studied. His temperature conditions were generally not those of maintained temperatures, though some tests were carried out in a cold-storage room at about 4° . For most of the tests he employed a definite type of fluctuating temperature involving what may be called alternating maintained temperatures; the cultures were transferred twice daily (morning and evening) from one incubator to another, two incubators (each with its own maintained temperature) being employed for any given test. The pairs of maintained temperatures employed were 2° and 10° , 10° and 18° , 18° and 27° , and 27° and 35° . Thus, for example, a culture was subjected to a maintained temperature of 18° during the day time, the temperature was then quickly changed to 10° and the culture was subjected to this lower maintained temperature overnight, the temperature being again quickly changed back to 18° on the following morning, etc. KORSTIAN's results are presented in graphs constructed similarly to those of BOERKER.

Although the present study was conducted along lines somewhat like those followed in KORSTIAN's work, the two plans were essentially different in many fundamental respects, as will appear later. Only maintained temperatures were employed in the present work, excepting as temperature fluctuation was necessitated by the short observation periods (when the cultures were outside of the incubators) and the background conditions were arranged so as to be reproducible with unusually close approximation. The problem, as set up in planning these studies, is much less complex than has generally been the case in earlier studies on the germinative energy of lots of seed and the environmental complexes for which the results are to be considered as valid are here perhaps more closely defined than has thus far been customary in this kind of experimentation.

The main features of this study of conifer seeds may be summarized as follows: The general or background conditions were considered with some care and a sort of standard technique was worked out for them. Light was excluded from the cultures excepting as they had to be removed from the culture chambers for observation. Many different maintained tempera-

tures were employed and many different lengths of the time period of incubation for each maintained temperature. To ascertain the percentages of germination for different environmental complexes and for different initial physiological or internal complexes (lots and kinds of seed) was the immediate aim of the experimentation. The work was planned primarily to advance our knowledge of principles or generalizations that may express the causal relations of maintained temperature and its duration to the percentage of germination occurring in the lots of seed tested; that is, to the germinative energy of each lot as manifested under the conditions of the several tests.

The study here reported was carried out between October, 1927, and March, 1928, in the Laboratory of Plant Physiology of the Johns Hopkins University, where the author occupied a position as student assistant. The work was made possible by a furlough granted the writer by the United States Department of Agriculture, which allowed him to be absent from his post with the Appalachian Forest Experiment Station during the academic years of 1926-27 and 1927-28. For himself as well as for the Johns Hopkins Laboratory of Plant Physiology, he wishes to mention with appreciative thanks the kindness of those who supplied the seeds used in the work. Their names are recorded on a subsequent page. For useful information about molds that were encountered in the cultures cordial thanks are expressed to Doctor CARL HARTLEY, who was once a student in this laboratory, to Mr. L. W. R. JACKSON, both of the United States Bureau of Plant Industry, and to Doctor CHARLES THOM, of the United States Bureau of Chemistry and Soils. The author wishes to express his thanks to Doctor W. H. TISDALE, of the Jackson Laboratory of the E. I. du Pont de Nemours Company, for supplying samples of the antiseptic "Semesan" and communicating valuable information about its composition and use. Finally, he desires to acknowledge his indebtedness to Doctor BURTON E. LIVINGSTON, director of the Laboratory of Plant Physiology of the Johns Hopkins University, for constant aid and criticism in the planning and conduct of the experiments, in the logical analysis of the results and in their presentation. He is also greatly indebted to his wife, BESSIE AMERMAN HAASIS, for helpful suggestion and criticism throughout the course of the work.

Experimental methods and materials

STANDARD CULTURES AND THEIR TREATMENT

The cultures of this study may be conveniently grouped in two categories, (1) the standard cultures and (2) a number of preliminary or supplemental additional cultures of other types. The standard cultures were all prepared and treated in the same general manner and they and their

treatment will be described first, after which the additional cultures and their treatment will be briefly described, largely as modifications of the standard type.

The seeds were germinated on agar plates in covered glass Petri dishes 9 cm. in diameter and 1 cm. deep, which had been held at a temperature of 140°–160° C. for at least an hour in an electric oven. Sufficient granulated agar ("Bacto-Agar," from the Digestive Ferments Co., Detroit, Michigan) for the entire investigation was thoroughly mixed before work was begun, to form a single stock of this material. Very close similarity in the many lots of agar solution that were to be prepared during the course of the experimentation was thus insured. A stock of "Semesan" (from the E. I. du Pont de Nemours Co., Wilmington, Delaware, described as containing 35 per cent., by weight, of hydroxy-mercuri-chloro-phenol, the balance being about one-third CaCO_3 and two-thirds Na_2CO_3) was prepared by mixing in the same way. The water used was from a "Barnstead" still.

The agar plates were prepared as in bacteriological procedure. The granulated agar was poured into the water while the latter was boiling and the mixture was stirred till all agar particles had disappeared. A gram of dry agar was used for each hundred cubic centimeters of water. Different lots of this mixture were made from time to time, for the several series of plates. Twenty cubic centimeters of the liquid agar mixture was used for each standard plate, being pipetted into the Petri dish while hot. Each dish was then immediately covered and allowed to cool.

The seeds, which had been previously soaked in antiseptic solution, were distributed uniformly on the surface of the agar plate, one hundred seeds to a plate. The seeds were arranged in a square pattern of ten rows, ten in a row, at distances of 6 mm. each way. Uniform spacing, secured by using a guide diagram or pattern on paper lying under the dish while the seeds were being distributed, obviated the necessity for tedious counting. The soaked seeds were placed by means of forceps, which were rinsed in standard "Semesan" solution from time to time while the work of distributing the seeds was in progress. The seeds quickly adhered to the agar gel sufficiently to allow the cultures to be freely handled and soon inverted without alteration in the arrangement pattern.

Because preliminary tests indicated that the seeds in an experiment were apt to become moldy before germination occurred, they were regularly soaked, for an hour just before being distributed on the plates, in a solution of "Semesan." This preliminary treatment was found to retard the development of mold. It should be noted that the antiseptic treatment introduces special considerations and may complicate to some extent the analysis of the experimental conditions. The treatment may possibly have resulted

in some cultures giving a somewhat lower percentage of germination than they would have given if the seeds had been soaked in distilled water, but the evidence at hand indicates that such chemical influence was never significant. Without the antiseptic treatment the influence of mold would have been very serious with some of the sets of test conditions.

The standard "Semesan" solution used for preliminary soaking was prepared with one gram of the powder for each 400 cc. of water. Since the proprietary product is stated to contain 35 per cent. by weight, of hydroxy-mercuri-chloro-phenol, this standard solution is taken to contain 0.0875 gm. of this compound in each hundred cubic centimeters. Fresh lots of solution were prepared at intervals as required and the solution was never used when more than five days old.

Most of the seeds tested failed to sink in the antiseptic solution and a special technique was consequently required for the preliminary soaking. Each batch of seeds was soaked in a large-mouth, cork-stoppered bottle, of 250 cc. capacity, nearly filled with solution so as to contain only about 1 cc. of air. During the soaking period the bottles were continuously rotated about a horizontal axis (three or four turns a minute), being so fixed that the long axis of each bottle remained inclined about ten degrees from the axis of rotation. By the rotating of the bottle and the quick movement back and forth of the air bubble adjacent to the seeds, these were kept in constant motion during the period of soaking. Each seed was thus continually bathed in solution and the adjacent solution was continually renewed.

About a thousand seeds were soaked for each series of cultures. At the end of the soaking period the wet seeds were spread out on the plane bottom of a glass dish and were then transferred, one by one, to the agar plates. Only apparently healthy seeds were used, but no attempt was made to select them for size, color, etc., the chief aim being to obtain similar groups of one hundred seeds each, so that all cultures of the same lot of seed might be comparable. Seven cultures were usually included in each series, one for each of the seven different maintained temperatures that were available.

The time required for the distribution of the seeds in a series of cultures was generally less than an hour, being shorter as experience and practice increased. Toward the end of the study the seeds for a series of seven cultures were generally placed on the plates in half an hour. Soaking and placing both occurred at room temperature, generally between about 19° and about 22° C. On account of evaporation, the seeds were probably a little cooler than the air when placed on the plates. It is thus seen that each experiment started by soaking all seeds in standard "Semesan" solution for from 1.5 to 2 hours, with temperatures around 20°. As soon as

the preparation of a series of cultures was completed the cultures were distributed in the maintained-temperature chambers, so that the differences between the temperatures began to be effective about 1.5–2 hours after the seeds were put to soak. Since germination of the seeds studied is relatively slow at temperatures around 20°, it may be supposed that the pre-germination processes had not progressed far during the time period devoted to soaking and distribution. The general consistency of the results obtained indicates that the latter would not have been notably different if the preliminary period had been shorter or if it had been characterized by lower temperatures or by smaller temperature fluctuations.

THE MAINTAINED TEMPERATURES

The battery of controlled-temperature chambers used in this study was described in its main essentials by LIVINGSTON and FAWCETT (13) and its operation was referred to by FAWCETT (6), for whose studies on the temperature relations of some parasitic fungi it was originally constructed. A number of investigations have been carried out with the aid of this series of chambers in the last twelve years. The apparatus was overhauled in the fall of 1926 and mechanical refrigeration was introduced at that time, to replace ice cooling, which had been used before. The ice-water tank is now filled with calcium chloride brine (about equal parts by weight of the commercial salt and water) and this solution is cooled by a mechanical refrigeration unit such as is commonly employed in home refrigerators. The form used has an electric motor that intermittently operates a piston compressor, sulphur dioxide is the refrigerant and the apparatus is cooled by flowing water.

The introduction of mechanical refrigeration has rendered more convenient the employment of temperatures below that obtained with melting ice, but the mechanical unit used has not been without occasional lapses and need for expert attention and it has perhaps not been, on the whole, more satisfactory than was the daily addition of ice, especially when it has been required that the temperature of the cooling tank should approach room temperature. The features requiring attention (not always at convenient times in an experiment series) have been the motor itself and the thermostatic switch. Only once, at the time of installation, has any notable escape of sulphur dioxide occurred and that accident might have done no harm had the apparatus been located elsewhere than in a greenhouse full of living plants, for of course no cultures were in the chambers at the time. It is suggested that a refrigeration apparatus of the absorption type, without motor or pump, may possibly prove in many cases to be more suitable for use in scientific experimentation than those forms that depend on

mechanical devices for compressing the refrigerant. The series of chambers is located in one of the greenhouse rooms of the laboratory because of the lack of other more suitable space and the air temperature of the room fluctuates considerably. A basement room, preferably with automatic temperature control to within a few degrees of fluctuation, would be preferable to the present location.

The seven culture chambers of this series are cylindrical (38 cm. in diameter and 38 cm. deep) and each opens above. They are arranged in a row, each being provided with its own water-jacket, which has a motor-driven stirrer. Supported from the top of each chamber is another cylinder, of thin, multi-perforated galvanized sheet iron, 28 cm. in diameter and 43 cm. high, with its floor about 2 cm. above the floor of the outer cylinder. The 5-cm. layer of air between the outer and inner cylinders is kept in motion by two vertical stirrer rods, each of which is bent upon itself to form an inverted U, the terminal portion of which operates to stir the water of the jacket. The water-jackets of adjacent chambers are separated by sheet-iron partitions, which prevent any movement of water from jacket to jacket but allow heat transfer by conduction. At each end of the series is a control tank with stirrers and each of these tanks is separated from the jacket of the adjacent chamber by a sheet-iron partition. One of the tanks (at the hot end of the series) contains water, heated by means of an electric heater with thermostatic control. The control tank at the other (cold) end of the series, which also has stirrers, contains calcium-chloride brine and has thus far been operated without heating apparatus. The sheet-iron partition between it and the cooling tank (where refrigeration is applied) has perforations which, in these studies, have been only partially closed by rubber stoppers, so that circulation of brine might occur between these two tanks. The thermostatic regulation of the temperature of the control tank at the cold end has thus been left to the refrigeration apparatus. The water of the chamber jackets and that of the hot control tank is covered by a thin layer of engine oil, to retard evaporation. Sides, bottom and ends of the series as a whole are insulated with a layer of tightly packed oxbair (such as is used in plaster for the interior of buildings) and the top, including the removable covers of the several chambers, is insulated by pine wood and about 3 cm. of sheet cork. The whole is covered above, in addition, by pads of saddler's hair-felt 3 cm. thick, two thicknesses of ordinary wool bed-blankets and a white canvas tarpaulin. In spite of this extra insulation for the tops of the chambers, it appears that most of the heat leakage occurs through this region. A considerable amount of heat leakage is of course inevitably due to the opening of the chambers for observations. Visible radiation is completely excluded from the chambers, and the cultures were therefore in darkness excepting when observation was necessary.

For any set of seven maintained temperatures (or of narrow temperature ranges, to be more precise) it is necessary only to adjust the two thermostats that control the supply and the removal of heat at the ends of the series. A day or two of idle operation follows each new setting, to allow the chambers to approach dynamic equilibrium with one another and with the control tanks, after which each one maintains its temperature indefinitely, generally with a fluctuation of less than a single centigrade degree above and below the mean. The temperature fluctuations of the cultures were of course greater in most instances, on account of their being removed at intervals for observation, but the periods of departure from the mean on this account were kept as short as possible.

The difference between the temperature of the water or brine in either control tank and the air temperature in the adjacent chamber was about 10° , being somewhat larger or smaller according to the settings of the thermostatic controls. The difference between the air temperatures of any two adjacent chambers was of course determined by the two control temperatures in any setting. It was not uniform throughout the series, being usually smaller toward the colder end.

Each chamber is provided with a calibrated thermometer of the ordinary chemical type, which may be momentarily raised and read without disturbing the chamber cover. Temperatures were observed and recorded at frequent intervals in each period of operation and small Richard thermographs were operated in some of the chambers, while maximum-minimum registering thermometers of the Six type were used in some cases. From the available temperature records a mean was derived as accurately as possible for each chamber for each period of operation, to represent the maintained temperature of that chamber for the period in question. The means for eleven periods of operation, considered as the respective maintained temperatures dealt with, are shown in table I, where the eleven periods are numbered chronologically and the seven chambers are numbered serially, beginning at the cold end of the series.

It will be seen from the data of table I that the same chamber was used, at different times, for different maintained temperatures, and that the same maintained temperature was in many instances obtained in different chambers at different times. No relation was discovered between the seed responses and the particular positions (in the greenhouse) of the chambers in which they occurred, and the use of different chambers for the same maintained temperature need not be considered further. Similarly, no relation was observed between the seed responses and the periods of operation in which they occurred; hence the temperature means for all periods of operation of the control chambers may be considered in a single series,

TABLE I
PERIODS OF OPERATION OF MAINTAINED-TEMPERATURE CHAMBERS AND MEAN TEMPERATURES OF THE SEVEN
CHAMBERS FOR EACH PERIOD

PERIOD OF OPERATION		TEMPERATURE MEANS						
PERIOD NO.	DATES (1927-28)	1ST CHAMBER	2ND CHAMBER	3RD CHAMBER	4TH CHAMBER	5TH CHAMBER	6TH CHAMBER	7TH CHAMBER
1	Oct. 14-26	Deg. C. 20	Deg. C. 25	Deg. C. 31	Deg. C. 35	Deg. C. 42	Deg. C. 48	Deg. C. 55
2	Oct. 31-Nov. 16	16	20	24	27	33	37	41
3	Nov. 16-18	18	21	26	29	34	37	42
4	Nov. 25-Dec. 8	26	29	33	36	41	45	51
5	Dec. 16-24	8	12	15	18	22	24	28
6	Dec. 29-Jan. 8	20	25	31	36	43	50	57
7	Jan. 11-24	20	24	28	32	36	41	46
8	Jan. 28-Feb. 4	23	26	30	35	39	44	50
9	Jan. 30-Feb. 11	26	28	31	36	40	45	50
10	Feb. 25-Mar. 9	18	22	26	30	36	39	45
11	Mar. 3-27	18	22	26	30	36	40	46

as though all tests had been made simultaneously. The following list, compiled from table I, shows the maintained temperatures actually employed, together with notations as to the corresponding periods and chambers. The period numbers, with subscripts to indicate chamber numbers, are shown in parentheses for each temperature.

8° (5 ₁)	33° (2 ₅ , 4 ₃)
12° (5 ₂)	34° (3 ₅)
15° (5 ₃)	35° (1 ₄ , 8 ₄)
16° (2 ₁)	36° (4 ₄ , 6 ₄ , 7 ₅ , 9 ₄ , 10 ₅ , 11 ₅)
18° (3 ₁ , 5 ₄ , 10 ₁ , 11 ₁)	37° (2 ₆ , 3 ₆)
20° (1 ₁ , 2 ₂ , 6 ₁ , 7 ₁)	39° (8 ₅ , 10 ₆)
21° (3 ₂)	40° (9 ₅ , 11 ₆)
22° (5 ₅ , 10 ₂ , 11 ₂)	41° (2 ₇ , 4 ₅ , 7 ₆)
23° (8 ₁)	42° (1 ₅ , 3 ₇)
24° (2 ₃ , 5 ₆ , 7 ₂)	43° (6 ₅)
25° (1 ₂ , 6 ₂)	44° (8 ₆)
26° (4 ₁ , 3 ₃ , 8 ₂ , 9 ₁ , 10 ₃ , 11 ₃)	45° (4 ₆ , 9 ₆ , 10 ₇)
27° (2 ₄)	46° (7 ₇ , 11 ₇)
28° (5 ₇ , 7 ₃ , 9 ₂)	48° (1 ₆)
29° (3 ₄ , 4 ₂)	50° (6 ₆ , 8 ₇ , 9 ₇)
30° (8 ₃ , 10 ₄ , 11 ₄)	51° (4 ₇)
31° (1 ₃ , 6 ₃ , 9 ₃)	55° (1 ₇)
32° (7 ₄)	57° (6 ₇)

No special attempt was made to control the air composition in the culture chambers nor in the culture dishes themselves. The air over the agar plate must have been generally nearly saturated with water vapor and the germination data indicate clearly that the supply of oxygen (or the disposal of carbon dioxide) was in no case a limiting condition. It should be noted that the covers of the culture chambers and those of the dishes were surely not tight enough to seriously retard gas diffusion and that the air of the chambers generally approached water saturation.

SPECIAL CULTURES AND THEIR TREATMENT

Besides the standard cultures, which were used in most of the tests, a number of preliminary and supplementary tests with modifications of the standard culture methods were employed. In some of these less than 100 seeds were used for each culture. In some instances dry seeds were placed on the plates without preliminary soaking, or they were soaked in tap-water or in distilled water instead of "Semesan" solution, or the concentration of "Semesan" and the period of soaking were different from that noted above, or some other antiseptic was employed.

For these special cultures the temperature chambers of the series described above were employed in some instances, and electric incubators were

used in other instances. The latter maintained mean temperatures of 25°, 30°, and 35°, with fluctuation of about one degree above and below the mean. A few cultures were transferred from the regular temperature chambers to incubators before observations were completed. Further details concerning these additional cultures will be presented farther on, as required in the presentation of the results.

OBSERVATIONS, RECORDS, COMPUTATIONS AND GRAPHS

Cultures were usually placed in the chambers in the forenoon, within an hour of the placing of the soaked seeds upon the agar plates, and observations were usually made 21 hours later and at 24-hour intervals thereafter. No effort was made to keep the observation intervals exactly uniform for all cultures of the same series and there was some variation in their length. Cultures were usually prepared and placed in the chambers on several successive days after the beginning of each period of operation. The several series of any period consequently overlapped in regard to time.

Observations were made on small groups of cultures (not more than six), and the rest remained in their chambers till work with the group in hand had been completed and they had been returned. The chambers remained closed excepting momentarily, when cultures were removed or replaced. Observations were made in the shade in the greenhouse, the work being performed as rapidly as possible. All cultures were consequently exposed to conditions other than those of the culture chambers for a short time whenever observations were made. As much as an hour and three quarters might elapse between the observation of cultures from the first chamber and the observation of cultures from the seventh chamber. Observation regularly began with the coldest chamber and proceeded along the series of chambers to the hottest one.

Observation intervals were not always the same for different series. Intervals of a few hours were used in a number of experiments. In a few cases different series were started at 2-hour intervals, so that observations made at about the same time might give data for intervals of several different lengths; *e.g.*, for 10, 12, and 14 hours from the beginning of the test. For experiments in which the observations of the first day or two were to occur at short intervals, the number of cultures was so restricted that the examination of all of them might be completed in fifteen minutes. It is obvious that the number, frequency and distribution of the observations made on a given culture in an experiment period must have largely determined the number and distribution of the pronounced temperature and other environmental fluctuations to which that culture was inevitably subjected on account of its temporary removal from the chamber for observation.

The Petri dish remained uncovered for a few minutes during each examination of each culture and all germinated seeds were removed with forceps and counted at each examination, the points of the forceps being frequently dipped in standard antiseptic solution. A satisfactory background was afforded by a piece of black paper placed beneath the culture dish during the examination.

The attainment of germination was considered as having occurred as soon as a definite and clearly discernible projection of the embryo had pushed out from the ruptured seed coat. For the conifer seeds here considered this implies the protrusion of the primary root. This is an easily observed stage or phase of the germination process, arbitrarily chosen for convenience. A similar stage was employed by KORSTIAN (10) in his study of germinating acorns. If it seems desirable the attainment of this protrusion stage of germination may be considered as marking with a considerable degree of precision the transition from germinating seed to seedling, but there is not as yet any generally-followed convention in the use of these vague terms. Perhaps many students of seed germination would consider that germination begins with the onset of swelling and that the process continues till leaves appear. However that may be, in the present account the word germination implies simply the attainment of the protrusion stage.

Since observation of any culture could not be continuous and since undesirable environmental fluctuations became more pronounced with more frequent observations, many seeds had progressed far beyond the critical protrusion stage before germination was noted. At the end of a 24-hour period seeds of pitch pine, for example, might exhibit primary roots as much as 5 mm. in length. The inaccuracies thus introduced always tend to render the recorded incubation period for a seed, or the recorded mean period for a number of seeds, too long; it is never too short. Account was taken of these sources of inaccuracy wherever possible. They generally vanish or become insignificant for our present purposes, through the averaging processes to which all numerical data were subjected.

The number of seeds that had germinated in each culture since the last observation was ascertained and suitably recorded for each observation, and the total number that had germinated since the beginning of the test was then obtained for each incubation period, by summation. For example, a certain standard culture incubated at a mean temperature of 32°, which had shown no seeds germinated at the end of the first day, showed 10 germinated at the end of the second day, 30 more at the end of the third day, and 19 more at the end of the fourth day. The percentages of germination for the four different incubation periods were therefore as follows:

Length of incubation period	1 day	2 days	3 days	4 days
Percentage of germination	0	10	40	59

Each standard series thus gave a set of seven values or indices of germinative energy for each incubation period used, one value for each maintained temperature. These indices were plotted to give a temperature-germination graph, with abscissas representing maintained temperatures and ordinates representing the percentage values, and each length of incubation period naturally had its own graph. From a system of such graphs it was easy to observe what was the temperature giving the highest percentage for each length of incubation period, what was the general form of each graph and how the graph form altered from one length of period to another.

When the experimentation had been discontinued all comparable data for each lot of seeds were assembled in a single tabulation and all indices of germinative energy were averaged for each of the several tested maintained temperatures and for each length of incubation period. For example, there were ten values for lot 1 of pitch pine seed for the 3-day incubation period and for the maintained temperature of 41°. These were obtained from three periods of operation of the temperature chambers, as follows: (1) three values from as many cultures in the fifth chamber, (2) two values from two cultures in the sixth chamber, and (3) five values from five cultures in the seventh chamber. The ten comparable values (which ranged in magnitude from 12 to 34) were averaged to give the final average index for this lot of seed for the 3-day incubation period and for the maintained temperature of 41°. The final average index is 20 in this case. An average index was obtained in this manner for each of the remaining temperatures that had been employed for the 3-day period and these were plotted to form a graph for that length of period and for this particular lot of seed. Other lengths of incubation period for the same lot of seed were treated in the same general way, each length of period having its own graph, and these graphs were assembled on one sheet, with the abscissas the same for all.

SEEDS USED

The lots of seed with which this paper is concerned are listed below.

Pitch pine (*Pinus rigida* Mill.) :—

Lot 1. Crop of 1926, New Jersey. (Received in June, 1927.^a)

Lot 2. Crop of 1926, New Jersey. (Received in December, 1927.^a)

Lot 3. Crop of 1927, New England. (Received in January, 1928.^a)

Lot 4. Crop of 1927, New Jersey. (Received in January, 1928.^a)

Lot 5. Crop of 1927, Franklin Co., Pennsylvania. (Received in February, 1928.^b)

Red pine (*P. resinosa* Ait.). Crop of 1927. (Received in December, 1927.^a)

Scotch pine (*P. sylvestris* L.). Crop of 1927. (Received in January, 1928.^a)

Lodgepole pine (*P. murrayana* Balf.). Crop of 1927. (Received in January, 1928.^a)

Long leaf pine (*P. palustris* Mill.). Crop of 1927. (Received in November, 1927.^a)

Western yellow pine (*P. ponderosa* Laws.). Crop of 1927, Hat Creek Forest, British Columbia. (Received in February, 1928.^b)

Loblolly pine (*P. taeda* L.). Crop of 1927, Pender Co., North Carolina. (Received in February, 1928.^b)

White spruce (*Picea canadensis* BSP). Crop of 1926. New England. (Received in June, 1927.^a)

Engelmann spruce (*P. engelmanni* Engelm.). Crop of 1926, Mt. Ida, British Columbia. (Received in January, 1928.^b)

Smooth cypress (*Cupressus glabra* Sudw.). Crop of 1927. (Received in November, 1927.^a)

Results and discussion

PITCH PINE SEED, LOT 1, STANDARD CULTURES

Data from standard cultures of lot 1 of pitch pine seed are assembled in table II, where each line is devoted to a single maintained temperature and each column is restricted to a single length of the incubation period. The number of cultures on which each mean germination percentage is based is shown as a subscript numeral following the mean value. These data are shown also by the nine narrow-line graphs of figure 1, there being a graph for each length of incubation period shown in the table. On these graphs abscissas represent temperature means, and ordinates represent percentages of germination. All the graphs are plotted on the same scale but with different base lines, the positions of the several different base lines being shown at the left of the figure (0_{6-7} hr. to 0_{14} da.). The temperature scale is shown at the bottom.

Examination of the graphs of figure 1 will make apparent the main contributions of the present paper. While the data on which the graphs are based are not as numerous and extensive as might be wished, yet they are generally consistent and their indications seem to be clear.

The maximal temperature was not reached; even incubation periods as short as 6 or 7 hours showed some seeds to have germinated with the highest

^a Purchased from C. B. FLEU, JR., Philadelphia.

^b Lots marked b were kindly furnished as follows: Pitch pine, lot 5, by Mr. J. S. ILLICK, Harrisburg, Pa. Western yellow pine, by Mr. A. C. THRUFP, Kamloops, B. C. Loblolly pine, by Dr. C. F. KORSTIAN, U. S. Forest Service. Engelmann spruce, by Mr. P. M. BARR, Victoria, B. C.

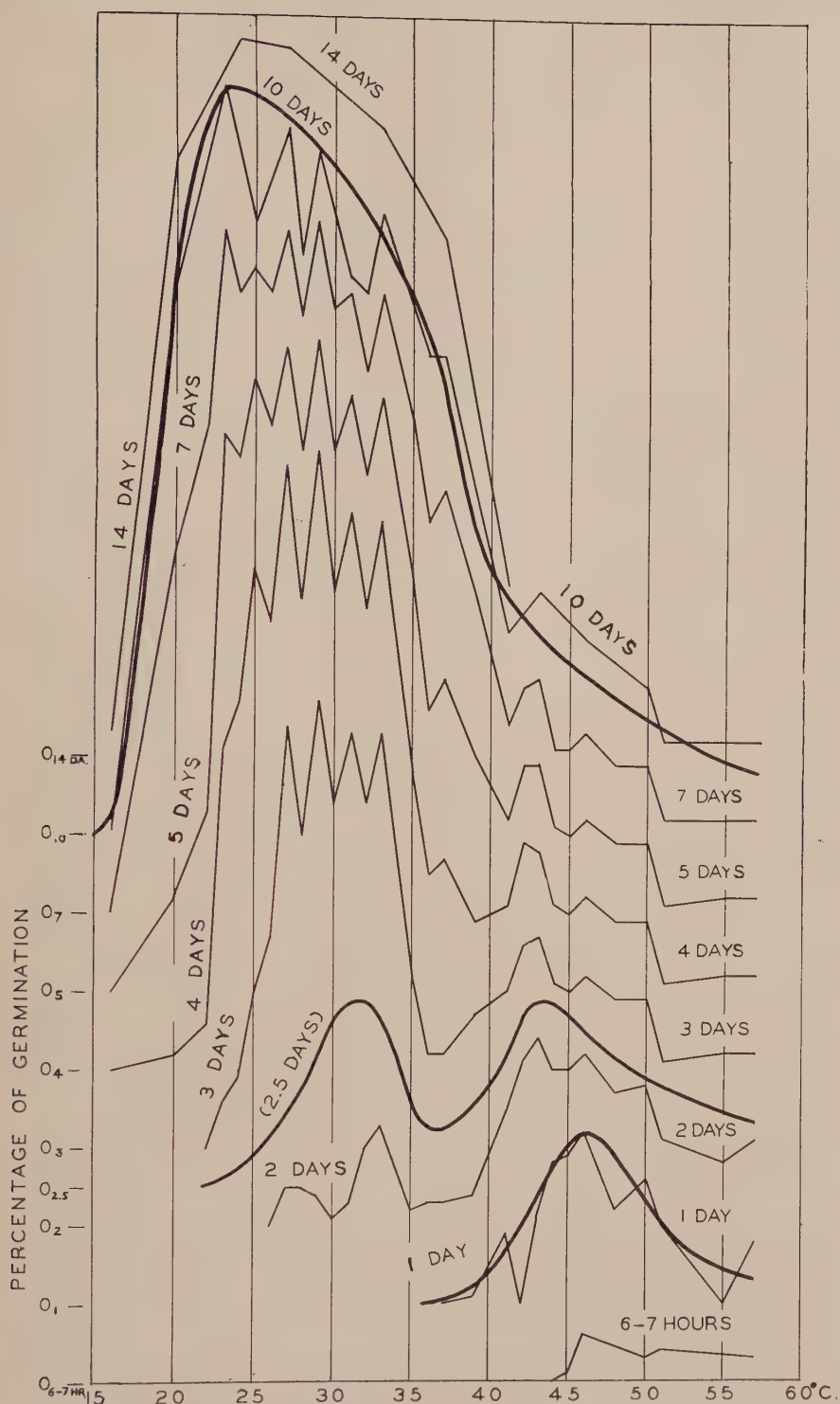


FIG. 1. Temperature-germination graphs for pitch pine seed, lot 1, for 6-7 hours, and for 1, 2, 3, 4, 5, 7, 10 and 14 days of incubation. The broad lines are smoothed graphs for the 1-day and 10-day periods and an interpolated graph for a period of incubation of about 2.5 days. Positions of the several different base lines are shown at the left, the subscript numerals indicating the respective lengths of incubation period.

TABLE II

MEAN PERCENTAGE OF GERMINATION (GERMINATIVE-ENERGY INDICES) OF SEEDS OF PITCH PINE, LOT 1, IN STANDARD CULTURE, FOR MAINTAINED TEMPERATURES BETWEEN 16° AND 57° AND FOR INCUBATION PERIODS OF FROM 6 OR 7 HOURS TO 14 DAYS. (THE NUMBER OF CULTURES ON WHICH EACH MEAN IS BASED IS SHOWN BY A SUBSCRIPT NUMERAL)

MAIN- TAINED TEMP.	PERCENTAGE OF SEEDS GERMINATED AT END OF								
	6-7-HR. PERIOD	1-DAY PERIOD	2-DAY PERIOD	3-DAY PERIOD	4-DAY PERIOD	5-DAY PERIOD	7-DAY PERIOD	10-DAY PERIOD	14-DAY PERIOD
Deg. C.									
16	—	0 ₅	0 ₅	0 ₅	0 ₅	0 ₅	0 ₅	1 ₄	3 ₂
20	0 ₃	0 ₁₀	0 ₁₀	0 ₁₀	2 ₁₀	12 ₁₀	46 ₁₀	70 ₆	76 ₂
22	—	0 ₂	0 ₂	0 ₂	0 ₂	23 ₂	62 ₂	—	—
23	0 ₁	0 ₄	0 ₄	6 ₄	41 ₄	71 ₄	87 ₄	95 ₂	—
24	0 ₂	0 ₆	0 ₆	9 ₆	47 ₆	68 ₆	79 ₆	87 ₃	91 ₂
25	0 ₁	0 ₄	0 ₄	20 ₄	64 ₄	78 ₄	82 ₄	78 ₁	—
26	0 ₁	0 ₂	0 ₂	27 ₂	57 ₂	72 ₂	79 ₂	—	—
27	—	0 ₅	5 ₅	54 ₅	77 ₅	82 ₅	87 ₅	90 ₄	90 ₂
28	0 ₂	0 ₇	5 ₇	40 ₄	60 ₄	69 ₄	76 ₄	74 ₁	—
29	—	0 ₂	4 ₂	57 ₂	79 ₂	83 ₂	88 ₂	87 ₁	—
30	0 ₁	0 ₁	1 ₁	44 ₁	61 ₁	69 ₁	77 ₁	—	—
31	0 ₁	0 ₃	3 ₃	53 ₃	71 ₃	76 ₃	79 ₃	71 ₁	—
32	0 ₂	0 ₂	10 ₂	44 ₂	59 ₂	66 ₂	69 ₂	69 ₁	—
33	—	0 ₁₀	13 ₁₀	53 ₇	70 ₇	76 ₇	79 ₇	79 ₅	80 ₂
35	—	0 ₂	2 ₂	22 ₂	38 ₂	53 ₂	63 ₂	—	—
36	0 ₃	0 ₉	3 ₉	12 ₄	25 ₄	36 ₄	50 ₄	61 ₃	—
37	—	0 ₅	3 ₅	12 ₅	27 ₅	40 ₅	54 ₅	61 ₄	66 ₂
39	0 ₁	1 ₁	4 ₁	17 ₁	19 ₁	30 ₁	41 ₁	—	—
41	0 ₄	9 ₁₅	15 ₁₃	20 ₁₀	21 ₁₀	22 ₁₀	24 ₁₀	26 ₅	22 ₁
42	—	0 ₁	21 ₁	26 ₁	29 ₁	29 ₁	29 ₁	—	—
43	0 ₁	11 ₁	24 ₁	27 ₁	28 ₁	29 ₁	30 ₁	31 ₁	—
44	0 ₁	18 ₁	20 ₁	21 ₁	21 ₁	21 ₁	21 ₁	—	—
45	1 ₂	19 ₈	20 ₆	20 ₃	20 ₃	20 ₃	21 ₃	—	—
46	6 ₂	22 ₂	22 ₂	22 ₂	22 ₂	22 ₂	23 ₂	25 ₁	—
48	—	12 ₁	17 ₁	19 ₁	19 ₁	19 ₁	19 ₁	—	—
50	3 ₂	16 ₂	18 ₂	19 ₂	19 ₂	19 ₂	19 ₂	19 ₁	—
51	4 ₂	10 ₈	11 ₆	11 ₃	11 ₃	11 ₃	12 ₃	12 ₁	—
55	—	0 ₁	8 ₁	12 ₁	12 ₁	12 ₁	12 ₁	—	—
57	3 ₁	8 ₁	11 ₁	12 ₁	12 ₁	12 ₁	12 ₁	12 ₁	—

maintained temperature employed (57°). For this lot of seed the absolute maximum temperature for germination was therefore surely considerably above 57°.

The minimal temperature for germination is shown to have been about 44° for the shortest period. For more prolonged incubation the minimum is shown to be progressively lower; about 38° for the 1-day period, about 26° for the 2-day period, and below 15° for the 14-day period. With still longer incubation the minimum would surely have appeared as well below, perhaps far below, 15°. To establish the absolute minimal temperature for the occurrence of germination in this lot of seed and for the background con-

ditions of these tests it would have been necessary to continue incubation much longer than fourteen days.

These observations on the temperature minimum emphasize at once the great importance of the duration factor in the consideration of this cardinal temperature. With seeds that germinate slowly it might be necessary to continue an experiment for many years before the absolute minimal temperature for germination could be ascertained. What percentage of the seeds of a lot can germinate at the absolute minimal temperature is another question, for answering which very prolonged incubation of the cultures would also be necessary.

Such considerations as these have been appreciated in a general way by those who have discussed minimal and maximal temperatures for physiological, and indeed for chemical and physical, processes, and it has frequently been noted that these two cardinal temperature values are usually capable of being ascertained only approximately. It may be remarked, however, that the maximum may always be stated as *above* a certain temperature (the highest temperature at which any germination occurred, for example), while the minimum may always be stated as *below* a certain temperature (the lowest at which the process referred to was observed).

With regard to optimal temperatures the graphs of figure 1 are especially interesting. For the shortest of the periods the optimum was only a degree or so above the corresponding minimum, being about 46° (6 per cent.). It was still about 46° (22 per cent.) for the 1-day period, but was somewhat lower, between 42° and 46° (21–24 per cent.), for the 2-day period. A second, and lower, optimum is clearly shown, however, for this same 2-day period of incubation, about 32° – 33° (10–13 per cent.), and an intermediate range of maintained temperatures between the two optima (from about 35° to about 39°) gave remarkably low percentages of germination (2–4 per cent.). For the 3-day and 4-day incubation periods the high-temperature optimum (26–27 and 28–29 per cent., respectively) was about 42° – 43° , but it had nearly disappeared for the 4-day period and is not evident for the longer periods represented in figure 1. The reason for the disappearance of the high-temperature optimum is clearly due to the fact that relatively few additional seeds germinated at about 42° – 43° after the end of the 3-day period, while larger numbers of additional seeds germinated at lower temperatures. The depression in the graph between the two optimal temperatures is progressively less pronounced for the 3-day and 4-day periods, and it fails to appear in the graphs for the 5-day and longer periods of incubation.

The second or low-temperature optimum (32° – 33°), shown as the smaller of the two in regard to percentage of germination for the 2-day period, is

represented by the highest percentage values on the graphs for the 3-day and longer periods. For the 5-day and longer periods, moreover, it is the only optimum and the graphs for these periods resemble, in form, many published graphs for the maintained-temperature relations of physiological processes.

For the 3-day period the low-temperature optimum (the one that appears as the only optimum for the longer periods, as just noted) is an optimal range, from about 27° to about 33° (40–57 per cent.); for the 4-day period this range is from about 25° to about 33° (57–79 per cent.); and for the 5-day and 7-day periods the optimal range (66–83 and 69–88 per cent.) is about the same as for the 4-day period. For the 10-day and 14-day periods the optimal temperature range appears to be narrower and located somewhat lower on the thermometer scale; for the longest incubation period represented, this range is from about 24° (91 per cent.) to about 27° (90 per cent.).

From these observations on the optimal temperatures it is very evident that the length of the period of incubation is a very important variable in the determination of the magnitude of the optimum and the characteristics of its range. The specification of this duration factor is even more essential in this connection than it is with regard to the temperature maximum and temperature minimum. As was emphasized by LEHENBAUER (11) and by FAWCETT (6), the magnitude of the temperature optimum for a process may, in general, be expected to vary considerably with the time factor, and statements as to temperature optima must usually be only vague and relatively unsatisfactory unless the length of the period of exposure is also given.

The importance of the length of the incubation period in the discussion of the characteristics of the temperature optimum for the occurrence of germination in this lot of seed is especially well shown when we consider the remarkable appearance of the double optimum for the 2-day and 3-day periods. It is evident that if these germination tests had been made with only 1-day, or with only 5-day or longer incubation periods, or if the periods used had been of several lengths but in every case less than 1 day or more than 4 days, no double temperature optimum would have been apparent from the numerical results and their graphs.

The three smoothed, broad-line graphs of figure 1 remain to be mentioned. The lowest of these is simply the smoothed graph for the 1-day period and the highest is the smoothed graph for the 10-day period. These give generalized pictures of the forms of the temperature-germination graphs for the short period (when the high-temperature optimum appears alone) and for the long period (when the low-temperature optimum is

alone evident). As the eye moves upward over the figure a continuous transition of the graph form, with progressively longer periods of incubation, is clearly indicated by the intervening narrow-line graphs, which represent the results of actual experiment. We may say that the lowest broad-line graph becomes gradually transformed as the incubation period is longer and longer, until it finally takes the form of the highest broad-line graph. In this continuous transformation the graph must pass through an intermediate form having two maxima with equal ordinates, each graph maximum representing one of the two temperature optima for germination. That particular stage in the graph transformation here envisaged is repre-

LENGTH OF PERIOD	MAXIMUM	HIGH-TEMPERA- TURE OPTIMUM (LARGE VALUES)	INTERMEDIATE TEMPERATURE (SMALL VALUES)	LOW-TEMPERA- TURE OPTIMUM (LARGE VALUES)	MINIMUM
6-7 hr.	57° +	46° (6 p. c.)	————	————	44°
1 da.	57° +	46° (22 p. c.)	————	————	38°
2 da.	57° +	42°-46° (21-24 p. c.)	35°-39° (2-4 p. c.)	32°-33° (10-13 p. c.)	26°
2.5 da.	57° +	43.5° (24 p. c.)	36°-37° (7 p. c.)	31.5° (24 p. c.)	
3 da.	57° +	42°-43° (26-27 p. c.)	36°-37° (12 p. c.)	27°-33° (40-57 p. c.)	22°
4 da.	57° +	42°-43° (28-29 p. c.)	39° (19 p. c.)	25°-33° (57-79 p. c.)	16°
5 da.	57° +	————	————	23°-33° (66-83 p. c.)	16°
7 da.	57° +	————	————	23°-33° (69-88 p. c.)	16°
10 da.	57° +	————	————	23°-29° (74-95 p. c.)	15°
14 da.	57° +	————	————	24°-27° (90-91 p. c.)	15°-

sented by the intermediate broad-line graph of the figure. This is not a smoothed graph for any series of experimental data actually available; it is a hypothetical graph, interpolated between the experimental graph for the 2-day period and that for the 3-day period, and it represents, as its parenthetical label indicates, an incubation period of approximately 2.5 days.

If the period of incubation represented by this interpolated graph had been employed in testing this lot of pitch pine seed the results would have been very striking indeed. For maintained temperatures about 31.5° and about 43.5° the percentage of germination would have been about 24, while it would have been only about 7 for a maintained temperature of about 36.5°.

To summarize the transition in graph form from that for the 6-7-hour period to that for the 14-day period, the cardinal features of the several graphs of figure 1 are tabulated, on p. 391. The interpolated data (for a 2.5-day period) are shown in black-face type.

The maximal temperature is above 57° . The minimal temperature recedes from about 44° (6-7-hr. period) to below 15° (14-day period). The high-temperature optimum recedes from about 46° (6-7-hr. period) to about 42° (4-day period) and then vanishes. The low-temperature optimum recedes from about 32° (2-day period) to about 25° (14-day period). The intermediate temperature, characterized by small percentage values, lies between 35° and 39° , being evident only for periods of from 2 to 4 days.

SPECIAL EXPERIMENTS WITH PITCH PINE SEED, LOT 1

Several special experiments with pitch pine seed of lot 1 were carried out with preliminary soaking in distilled water instead of in "Semesan" solution, standard procedure being otherwise employed. The results of these are shown by the graphs of figure 2, which show temperature optima for about 32° and about 41° , for incubation periods of 2 and 3 days, respectively. For the 10-day period a single optimum is shown as the temperature range from below 20° to about 32° (with germination percentages of

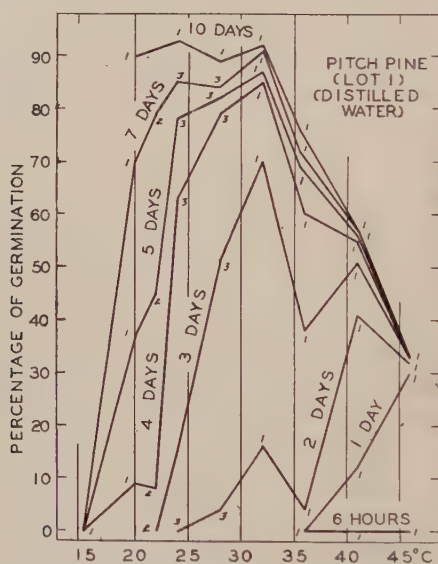


FIG. 2. Temperature-germination graphs for pitch pine seed, lot 1, preliminarily soaked in distilled water instead of "Semesan" solution, for 1, 2, 3, 4, 5, 7 and 10 days of incubation. No germination in 6-hour period. Numerals adjacent to points each indicate number of tests on which corresponding percentage value is based.

from 89 to 93). The temperature maximum is shown as at least above 46° and the minimum appears as about 36° for the 1-day period, about 24° for the 2-day period, about 22° for the 3-day period, and about 15° for the 4-day, 5-day, and 7-day periods. The numerals near the points on the graphs indicate how many cultures were used in each case.

A special experiment in which pitch pine seed of lot 1 was used without preliminary soaking at all, the dry seeds being distributed on the agar plates while the other details of the procedure were standard, showed for a 3-day incubation period one optimum about 30° (42 per cent.) and another for the temperature range from about 40° to about 46° (34 per cent.).

The double temperature optimum exhibited by the standard cultures of lot 1 of pitch pine seed is therefore not to be considered as related specially to the standard preliminary soaking in the antiseptic solution.

It seems clear that the recession of the minimal temperature with prolonged incubation and also the appearance of two separate optimal temperatures with intermediate lengths of the incubation period are related to an innate variability in the lot of seed. Apparently a sample of lot 1 of pitch pine consisted of several groups or categories of seeds, separable by experimentation of the type used in these studies. Turning again to the graphs of figure 1, it is evident that some of the seeds of each sample were capable of germination with an incubation period of 6 or 7 hours while some seeds failed to germinate at any temperature even with a 14-day period of incubation. If we regard the several cultures, of one hundred seeds each, as representative samples of the entire lot, about 20 per cent. of the lot were apparently capable of germination in a single day at a maintained temperature of 45° – 46° , while none were able to germinate in a period of this length at any maintained temperature below 37° . With prolonged incubation (10–14 days) only about 30 per cent. of the seeds could germinate at the high-temperature optimum (42° – 46°), but at the low-temperature optimum (24° – 25°) 90 per cent. of them germinated in the longest incubation period. Most of the seeds characterized by capacity to germinate at the higher temperatures were also characterized by capacity to germinate *quickly* at those temperatures, but these same seeds were apparently capable of germination at the low-temperature optimum if incubation was sufficiently prolonged.

The seeds of this lot 1 may be tentatively considered as roughly grouped in three physiological categories according to their germinative capacity in regard to maintained temperature, as shown for the 14-day period of incubation: (A) those capable of germination at about 25° but not capable of germination at about 43° , (A') those capable of germination at about 43° as well as at about 25° and (B) those not shown as viable at any maintained

temperature. The categories are not sharply distinguished but it appears that about 65 per cent. of this lot of seed belonged to the first category (A), about 25 per cent. to the second category (A') and about 10 per cent. to the third category (B). With the third of these hypothetical groups we need not be concerned in the present connection, but the first two groups (A and A') may be studied somewhat more closely by means of some available experimental data.

Many seeds that had germinated at 50° remained alive but grew only very slowly for as long as 10 days when kept at that temperature. If seeds that had germinated at about 50° were transferred, shortly after germination, to a temperature about 31° none of the seedlings grew well at the lower temperature and some were apparently dead at the time of transfer. Seeds that had germinated at about 43° and had then been immediately transferred to 31° showed seedling growth for 10 days, but the seedlings were apparently unhealthy. On the other hand, seedlings that had been produced at 31° and had then been transferred to 57° were dead within 8 days. In these special tests, the only seedlings that were growing well after 8 or 10 days were those that had been produced at about 31° and had been kept at that temperature.

These results indicate that, although about 25 per cent. (group A') of the seeds of this lot of pitch pine were capable of germination at the high-temperature optimum, the resulting seedlings failed to develop satisfactorily with such a high temperature and were, indeed, likely to grow very poorly even at lower temperatures.

Other transfers were made from one maintained temperature to another to gain evidence as to whether the higher temperatures had killed the seeds that had failed to germinate quickly; that is, whether seeds of category A were killed by the high temperatures that produced the prompt germination of those of category A'. Cultures that had been exposed for 2 days or more to a temperature of 45° or 51° (in which, respectively, about 20 per cent. or about 10 per cent. of the seeds had germinated at the higher temperature) failed to show any additional germination in a 7-day period after they had been transferred to a temperature of 25° or 30°. In a 100-seed culture transferred from 45°, of which 20 per cent. of the seeds had germinated in 25 hours, 12 additional seeds had germinated at the end of the following 7-day period at 25°. In a culture transferred from 51°, of which 10 per cent. of the seeds had germinated in 25 hours, only one additional seed germinated in the subsequent 7-day period at 25°. Of seeds that had been first incubated at a temperature of 41° for 7 or 9 days (in which time about 15 per cent. or about 20 per cent. of them had germinated), only one or two more germinated at 25° in the next seven days.

Cultures that had been incubated at 36° for 7 days (with germination of about 50 per cent.) or for 10 days (with a somewhat greater percentage of germination) were transferred to 25°, after which many additional seeds germinated, the total percentage of germination 7 days after the change being 80 or 85, about as great as was shown by corresponding cultures transferred from a temperature of 33° to one of 25°. Cultures initially incubated at 37° for from 11 to 15 days showed a slight acceleration in the rate at which additional germination occurred after they had been transferred to a temperature of 25°.

Cultures initially incubated for 7 or 10 days at 25°, 29° or 33° continued to show additional germination after being transferred to 25°. Cultures initially incubated at 16° for 15 days, with a germination percentage of only about 5, showed total percentages of 75 or 95, 3 days after transfer to 25° or 30°.

From the results of these special experiments it appears that when pitch pine seeds of this lot, in standard cultures, were incubated at a high maintained temperature (about 45°–50°) for more than about 2 days, those that had not already germinated in that time at the high temperature had lost their capacity for germination, even at a temperature of about 25°. On the other hand, when seeds that had failed to germinate in 15 days at a low temperature (16°) were transferred to a temperature of 25° or 30°, germination was very prompt and general, and the total percentage of germination at the end of an additional 7 days was about 90. It is therefore apparent that the seeds of category A (capable of germination at 25° but not at 43°) were generally killed by incubation at the higher temperature for a period long enough to produce germination, at that temperature, of the other viable seeds of the same lot (category A'). Seeds capable of germination about the high-temperature optimum germinated there very quickly but produced apparently unhealthy seedlings. These seedlings died if kept at the temperature that produced them and they developed only slowly when transferred to the low-temperature optimum shortly after they had germinated at the high-temperature optimum.

It is of course conceivable that seeds capable of germinating at the higher optimal temperatures might be able to germinate at even higher temperatures and perhaps even more quickly. If that were true a triple optimal temperature for the occurrence of germination might appear. In the present study no temperatures above 57° were tested.

OTHER LOTS OF PITCH PINE SEED

Graphs for the results given by standard cultures of lots 2, 3, 4 and 5 of pitch pine seed are shown in figures 3 to 6, which are plotted in a manner similar to that adopted for the graphs of figures 1 and 2. The number

of tests (cultures) upon which each percentage value is based is shown in each case by a numeral adjacent to the corresponding point on the graph. The numbers are small in all cases and these experimental results are therefore less well established than are those for the pitch pine seed of lot 1.

For lot 2 (fig. 3) the percentage values are markedly lower than the

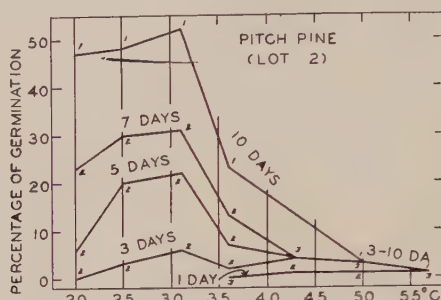


FIG. 3. Temperature-germination graphs for pitch pine seed, lot 2, for 1, 3, 5, 7 and 10 days of incubation. Numerals adjacent to points as in fig. 2.

corresponding indices for lot 1, for all of the maintained temperatures tested and for all the different lengths of incubation period for which data are available. A high-temperature optimum is indicated for the 1-day period and a double optimum (about 31° and about 43°) appears for the 3-day period. The maximum is shown as about 57°. The minimum is perhaps about 36° for the 1-day period and about 20° for the 3-day period. The low-temperature optimum is the range from about 25° to about 31° for the 5-day and for the 7-day periods for which it is the only optimum shown. For the 10-day period this range appears as from about 20° to about 31°.

For lot 3 (fig. 4) no indication of a double optimum appears. The

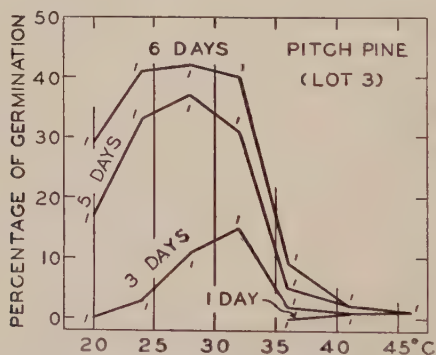


FIG. 4. Temperature-germination graphs for pitch pine seed, lot 3, for 1, 3, 5 and 6 days of incubation. Each percentage is based on but a single culture.

maximum temperature is indicated as above 45° . The minimum is about 36° for the 1-day period and about 20° for the 3-day period. The single (low-temperature) optimum is shown as about 32° for the 3-day period and it appears to be the range from about 24° to about 32° for the 5-day and 6-day periods.

For lot 4 (fig. 5) a high-temperature optimum is indicated for the 1-day

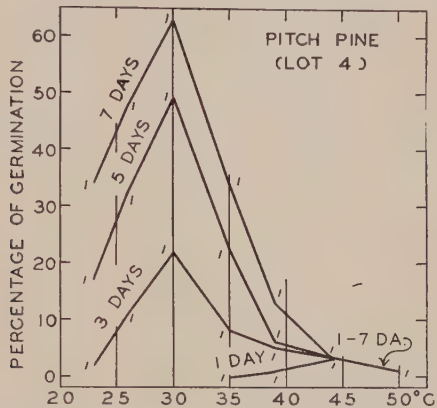


FIG. 5. Temperature-germination graphs for pitch pine seed, lot 4, for 1, 3, 5 and 7 days of incubation. Each percentage is based on but a single culture.

period (about 44°) and a low-temperature optimum appears about 30° for the 3-day, 5-day and 7-day periods, but no double optimum is actually shown. The maximum appears as above 50° and the minimum is shown about 35° for the 1-day period and below 23° for the 3-day period.

For lot 5 (fig. 6) a high-temperature optimum about 45° or above is

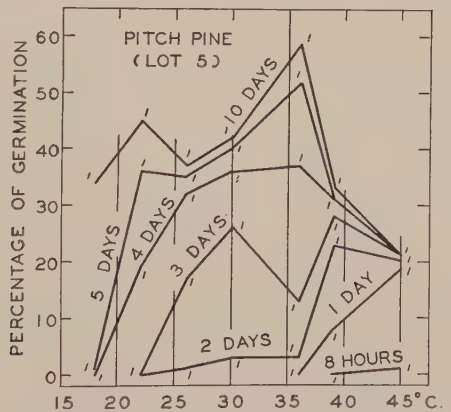


FIG. 6. Temperature-germination graphs for pitch pine seed, lot 5, for 8 hours and for 1, 2, 3, 4, 5 and 10 days of incubation. Each percentage is based on but a single culture.

suggested for incubation periods of 8 hours and 1 day and a similar optimum about 39° is shown for periods of 2 and 3 days. A low-temperature optimum (about 30°) appears with the other for the 3-day period, thus giving a double optimum for that period, with two nearly equal germinative-energy indices. For the longer periods of incubation that were tested the single optimum appears about 36° . The maximum is not indicated (excepting that it is shown as above 45°) and the minimum appears about 39° for the 8-hour period, about 36° for the 1-day period, about 22° for the 2-day and 3-day periods, about 18° for the 4-day period and below 18° for the 5-day and 10-day periods.

In general, the results of these standard tests with lots 2, 3, 4 and 5 of pitch pine seed indicate that lot 1 was peculiar in several respects, but they show that the double optimum so clearly established for seed of that lot may be expected from tests of other lots of this species, though exceptions are to be expected also. It appears that this phenomenon of a double optimum is related to differences (variability) in the internal conditions or physiological characteristics of the seeds of each lot for which it was exhibited. These differences and their distribution in the lot must have acted together with the environmental background conditions of the standard treatment. It does not seem desirable to attempt here to make suggestions as to precise and detailed reasons for the differences between these lots of pitch pine seed, although a number of apparently plausible hypotheses might be imagined. With the meager data at hand it is best to avoid any attempt in that direction and to say simply that the double optimum was shown for some lots of pitch pine seed in standard cultures for relatively short periods of incubation, while it failed to become evident in similar tests of other lots of seed of the same species. If a little deeper but very general analysis is desired, it seems to be safe to say: (1) that the individual seeds of each lot differed among themselves in their capacity to germinate under the several different sets of experimental conditions used in the tests, each set of conditions embracing, besides the standard background complex, one particular maintained temperature and one length of the incubation period; (2) that the seeds of any lot may be conveniently classified in a number of physiological categories according to their individual capacities for germination under the influence of the several experimental conditional complexes; and (3) that each lot was characterized by its own set of proportions of the seeds belonging to the several categories. The graphs of germinative energy as related to the duration of incubation and to temperature furnish pictures of the ways in which the lots differed in statistical make-up. According to these pictures the several lots might themselves be grouped in lot categories.

SEEDS OF OTHER CONIFEROUS TREES

Results of some less extensive experiments with lots of seed of the other coniferous trees dealt with in this study are summarized by the graphs of figures 7-15. The experimental procedure was standard for all but loblolly pine (fig. 9), longleaf pine (fig. 10), western yellow pine (fig. 12) and one series of Engelmann spruce (fig. 13, broken lines). In the case of loblolly pine only 50 seeds were used per culture and the preliminary soaking was in distilled water. In the case of longleaf pine only 25 seeds were used per culture, the procedure being otherwise standard. For Engelmann spruce, the series represented by broken lines in figure 13 differed from the standard only in that the preliminary soaking was in distilled water. The cultures of western yellow pine were standard excepting that only 50 seeds were used for each.

The experimental data for these nine species are in every case much less extensive than those for pitch pine seed, lot 1, and they are to be regarded as not very significant; but several of these series are clear in their indications concerning the double optimal temperature for germination. The graphs for these series are constructed like those previously presented and do not require special discussion, but the following notes on them are of interest.

For red pine (fig. 7) two temperature optima (about 24° and about 36°)

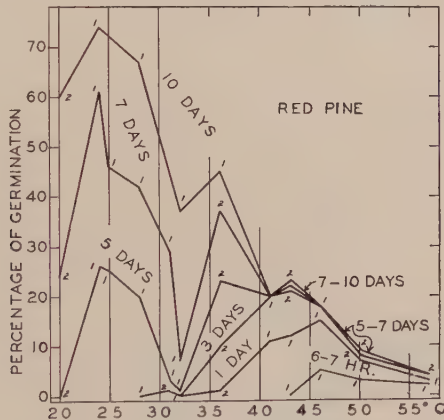


FIG. 7. Temperature-germination graphs for seeds of red pine, for 6-7 hours and for 1, 3, 5, 7 and 10 days of incubation. Numerals adjacent to points as in fig. 2.

are evident for incubation periods of 5, 7 and 10 days. For the two shorter periods tested (6-7 hours and 1 day) only a single optimum is shown (about 46°).

For lodgepole pine (fig. 8) two optima (about 24° and about 40°) appear for incubation periods of 3, 5, 7 and 10 days, and but one optimum

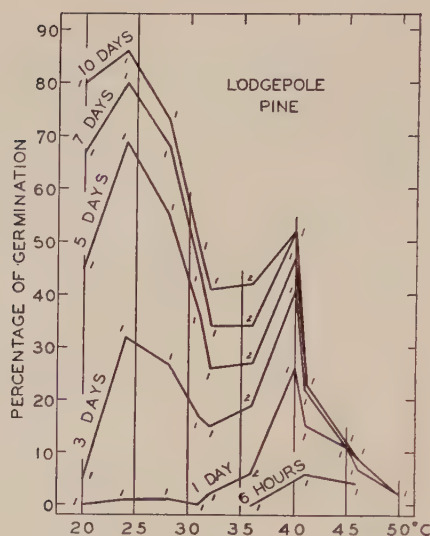


FIG. 8. Temperature-germination graphs for seeds of lodgepole pine, for 6 hours and for 1, 3, 5, 7 and 10 days of incubation. Numerals adjacent to points as in fig. 2.

(about 40° – 41°) is indicated for the two shorter periods (6 hours and 1 day).

For loblolly pine (fig. 9) two optima (about 26° and about 40°) appear

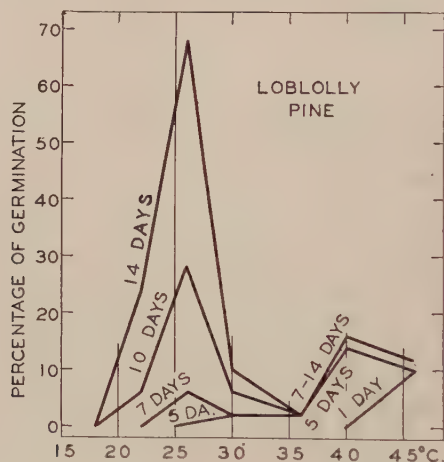


FIG. 9. Temperature-germination graphs for seeds of loblolly pine (50 seeds to the culture and preliminary soaking in distilled water instead of antiseptic solution), for 6.5 hours and for 1, 5, 7, 10 and 14 days of incubation. Each percentage is based on but a single culture.

for 7, 10 and 14 days. A single optimum is indicated for the 1-day period as above 46° , while for the 5-day period it appears to be about 40° .

For longleaf pine (fig. 10) two optima (apparently below 18° and above 46°) are indicated for incubation periods of 5, 7 and 10 days. For periods of 1 day and 3 days a single optimum is indicated as above 46° .

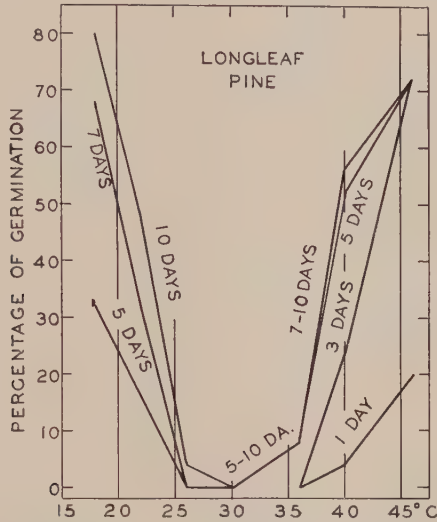


FIG. 10. Temperature-germination graphs for seeds of longleaf pine (25 seeds per culture), for 6 hours and for 1, 3, 5, 7 and 10 days of incubation. Each percentage is based on but a single culture.

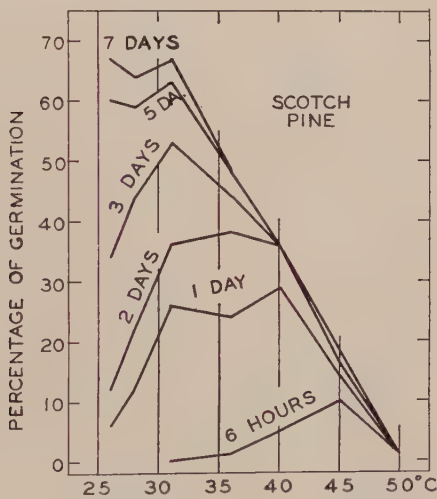


FIG. 11. Temperature-germination graphs for seeds of Scotch pine, for 6 hours and for 1, 2, 3, 5 and 7 days of incubation. Each percentage is based on but a single culture.

It was not until the tenth day of incubation that any seed germinated with a maintained temperature of 26° , and no germination had yet occurred at 30° when the experiment was discontinued after the cultures had been incubated 10 days. After the seventh day no additional seeds germinated with any temperature above 26° .

For Scotch pine (fig. 11) only a single optimum is indicated; about 45° for the 6-hour period, about 40° for the 1-day period, about 36° for the 2-day period, about 31° for the 3-day and 5-day periods and between 26° and 31° for the 7-day period. Two optima (about 31° and about 40°) are perhaps suggested for the 1-day period. A period of about 18 hours might have shown two optima, if such a period had been tested. It may be noted that SCHIMPER (20, page 46) quotes DETMER as stating that the maximal temperature for the germination of seed of Scotch pine is 34° , while the optimal temperature is 27° .

For western yellow pine (fig. 12) only a single optimum is shown;

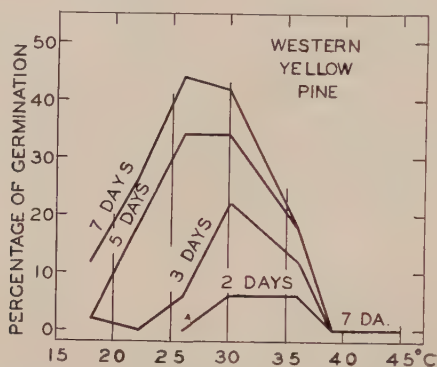


FIG. 12. Temperature-germination graphs for seeds of western yellow pine (50 seeds per culture), for 2, 3, 5 and 7 days of incubation. Each percentage is based on but a single culture.

between 30° and 36° for the 2-day period, about 30° for the 3-day period and between 26° and 30° for the 5-day and 7-day periods. It is possible that two optima might have appeared for the 2-day period if suitable maintained temperatures between 30° and 36° had been tested.

For Engelmann spruce (fig. 13) there were two series, for one of which the cultures had standard treatment while the seeds used in the other series had been soaked in distilled water instead of the standard antiseptic solution. In figure 13 the graphs for the standard tests are full lines and those for the special tests are broken lines. Two optima are clearly indicated (about 26° and about 40°) for periods of 3 and 5 days, also (about 26° and about 36°) for the 7-day period. A single high-temperature optimum

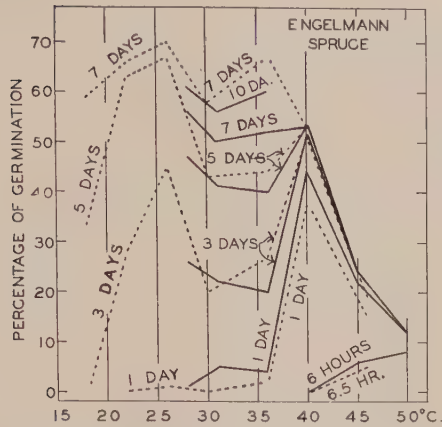


FIG. 13. Temperature-germination graphs for seeds of Engelmann spruce for 6-6.5 hours and for 1, 3, 5, 7 and 10 days of incubation. Solid lines represent standard cultures and broken lines represent cultures of seeds preliminarily soaked in distilled water instead of antiseptic solution. Each percentage is based on but a single culture.

(about 40°) appears for the 1-day period and the same is true (perhaps about 50° or even higher) for the period of 6 or 6.5 hours. As in the case of lot 1 of pitch pine seed, a satisfactory general agreement is shown between the results of the standard cultures and those of the tests with water-soaked seeds.

For white spruce (fig. 14) only a single optimum is indicated; about

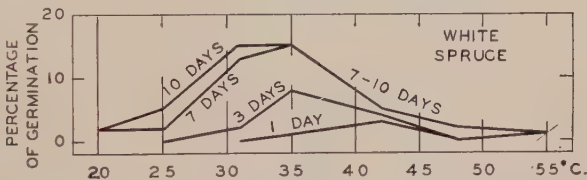


FIG. 14. Temperature-germination graphs for seeds of white spruce, for 1, 3, 7 and 10 days of incubation. Each percentage is based on but a single culture.

42° for the 1-day period, about 35° for the 3-day and 7-day periods, and between 30° and 35° for the 10-day period.

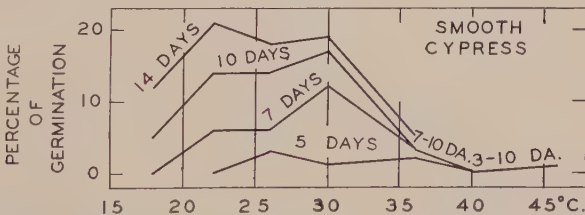


FIG. 15. Temperature-germination graphs for seeds of smooth cypress, for 3, 5, 7, 10 and 14 days of incubation. Each percentage is based on but a single culture.

For smooth cypress (fig. 15) two optima (about 26° and about 36°) are suggested for the 5-day period, but only a single optimum (about 30°) is indicated for the 7-day and 10-day periods, while two optima (about 22° and about 30°) are suggested for the 14-day period.

RÉSUMÉ OF RESULTS ON TEMPERATURE OPTIMA, AND ADDITIONAL NOTES

For convenience of reference and for comparison the main indications about optimal temperatures for the occurrence of germination in the several lots of seed, as set forth in the preceding sections of this paper and by the graphs of figures 1 to 15, are brought together in table III, the arrangement of which is self-explanatory.

There appears to be no doubt that the double optimum observed for lot 1 of pitch pine seeds is to be expected not only for other lots of seed of that species, but also for lots of seed of five other coniferous trees (red pine, lodgepole pine, loblolly pine, longleaf pine and Engelmann spruce). This expectation is suggested for Scotch pine and smooth cypress also. In some cases the double optimum is indicated for a wide range of lengths of the incubation period while in other cases it is confined to certain intermediate periods of incubation if these are neither too long nor too short.

In connection with the remarkably high maximal temperatures indicated for most of the lots of seed dealt with in this paper (50° or above, and apparently as high as 60° for pitch pine seed of lot 1), it may be remarked that seeds in nature may be temporarily subjected to temperatures above 60° for limited periods of time, as during hot sunny periods, if they lie on or near the soil surface. This may well be of frequent occurrence in the case of some species of pine that grow in areas subjected to high temperature and intense sunshine. For example, BATES (1) observed occasional temperatures of 66° C. about 0.5 cm. below the soil surface on south slopes in the central Rocky Mountain region, and TOUMEY and NEETHLING (23) record an extreme soil-surface temperature of 67° C. for New Hampshire. When such high soil temperatures occur they are apt to be confined to short periods (perhaps less than 12 hours) but they may be repeated for several days in succession or at longer intervals. It seems doubtful that moisture conditions suitable for seed germination are apt to be present at or near the soil surface when these very high temperatures occur.

In nature germinating seeds are almost always subjected to fluctuating temperature rather than to maintained temperature and the possible ecological bearing of the influence of temperature fluctuations on plant activities can not be approached seriously until much laboratory experimentation and precise analysis have been devoted to the general physiological problem of the influence of fluctuations in temperature as these affect the funda-

TABLE III

SUMMARY OF DATA ON OPTIMAL TEMPERATURES FOR THE OCCURRENCE OF GERMINATION IN THE SEVERAL LOTS OF SEEDS STUDIED, DERIVED FROM TABLE II AND FIGURES 1-14

KIND OF SEED AND TREATMENT	FIGURE NO.	LENGTH OF INCUBATION PERIOD	OPTIMAL TEMPERATURE		
			DOUBLE		SINGLE
			Low	High	
Pitch pine, lot 1 (standard cultures)	1	6-7 hr.	Deg. C.	Deg. C.	Deg. C.
		1 da.	—	—	46
		2 da.	32-33	42-46	46
		3 da.	27-33	42-43	—
		4 da.	25-33	42-43	—
		5-7 da.	—	—	23-33
		10 da.	—	—	23-29
		14 da.	—	—	24-27
Pitch pine, lot 1 (soaked in distilled water)	2	1 da.	—	—	46
		2-3 da.	32	41	—
		4-5 da.	—	—	32
		7 da.	—	—	24-32
		10 da.	—	—	20-32
Pitch pine, lot 2 (standard cultures)	3	3 da.	31	43	—
		5-7 da.	—	—	25-31
		10 da.	—	—	20-31
Pitch pine, lot 3 (standard cultures)	4	3 da.	—	—	32
		5-6 da.	—	—	24-32
Pitch pine, lot 4 (standard cultures)	5	1 da.	—	—	44
		3-7 da.	—	—	30
Pitch pine, lot 5 (standard cultures)	6	8 hr.	—	—	45
		1 da.	—	—	45
		2 da.	—	—	39
		3 da.	30	39	—
		4 da.	—	—	30-36
		5-10 da.	—	—	36
Red pine (standard cultures)	7	6-7 hr.	—	—	46
		3 da.	—	—	46
		5-10 da.	24	36	—
Lodgepole pine (standard cultures)	8	6 hr.	—	—	41
		1 da.	—	—	40
		3-10 da.	24	40	—
Loblolly pine* (soaked in distilled water) ^a	9	1 da.	—	—	46+
		5 da.	—	—	40
		7-14 da.	26	40	—
Longleaf pine* (standard cultures) ^b	10	1-3 da.	—	—	46+
		5-10 da.	18-	46+	—
Scotch pine* (standard cultures)	11	6 hr.	—	—	45
		1 da.	31(?)	40(?)	40(?)
		2 da.	—	—	36
		3-5 da.	—	—	31
		7 da.	—	—	26-31(?)
			—	—	

See next page for footnotes.

TABLE III—*Continued*

KIND OF SEED AND TREATMENT	FIGURE NO.	LENGTH OF INCUBATION PERIOD	OPTIMAL TEMPERATURE		
			DOUBLE		SINGLE
			LOW	HIGH	
Western yellow pine (standard cultures)	12	2 da.	—	—	30–36
		3 da.	—	—	30
		5–7 da.	—	—	26–30
Engelmann spruce* (standard cultures)	13	6 hr.	—	—	50 +
		1 da.	—	—	40
		3–7 da.	27 –	40	—
Engelmann spruce* (soaked in distilled water)	13	6.5 hr.	—	—	46 +
		1 da.	—	—	40
		3–5 da.	26	40	—
		7 da.	26	36	—
White spruce (standard cultures)	14	1 da.	—	—	42
		3–7 da.	—	—	35
		10 da.	—	—	31–35
Smooth cypress* (standard cultures)	15	5 da.	26 (?)	36 (?)	—
		7–10 da.	—	—	30
		14 da.	—	—	22 (?)

^a 50 seeds per culture.

^b 25 seeds per culture.

* A plus or minus sign after a number indicates that the number is too small or too large, respectively. An interrogation point in parentheses after a number indicates that the magnitude is questionable.

mental plant processes. Such investigation must, in turn, wait for much further advance in our knowledge of the influences exerted on plants by maintained temperature, a subject that has hardly yet begun to receive attention. The whole subject of temperature influence upon organisms constitutes a practically uncultivated field, the problems of which become more complex with each small contribution. Very fundamental physiological research will be needed before these problems may be subjected to sufficiently searching analysis to permit of the planning of experimentation by which they may possibly be simplified and rendered directly approachable.

The phenomena of double temperature optima for the occurrence of seed germination disclosed by the results of the present study are of peculiar interest because of their novelty and they may prove to be of great importance in the study of physiological temperature relations. The possible occurrence of two maintained-temperature optima for germination in a lot of seed is established by the present report, but the relation of these phe-

nomena to plant species and to lots of seed other than those here dealt with, as well as to other influential environmental complexes than those here tested, remains to be taken up. Seed of some species may not exhibit these phenomena at all while that of other species may exhibit them in some lots of seed but not in every lot. The fact that a double temperature optimum for the occurrence of germination has not been discovered for a given kind or lot of seed does not, of course, preclude the possibility that this characteristic may be disclosed in other lots of the same sort of seed or in lots of other sorts. The great significance of the length of the incubation period has been emphasized in earlier sections, and experimentation by which the occurrence of double temperature optima is to be sought must therefore be planned to embrace a number of different lengths of period as well as a number of different maintained temperatures. And the values for both of these variables must be so chosen as to cover the logical field adequately. The lengths of incubation period employed should differ serially by relatively small steps and the same is true of the maintained temperatures used. The range of period length and that of temperature must of course be broad enough to allow the double optimum to become manifest if the lot of seed in question should possess the capacity for exhibiting it. Finally, the environmental background complex employed in such studies as are here suggested (including all influential external conditions excepting temperature and duration) must surely be highly important in the determination of the occurrence or non-occurrence of evidence of a double optimum in any series of tests. It may be surmised that water relations and oxygen relations might furnish limiting conditions in many instances and the mind staggers at the thought of the infinite array of other possibilities suggested by such expressions as chemical influences, and chemical stimulation.

From studies of very different types, double temperature optima have been reported by KOEPPEN (9), for elongation of shoots, and by YAMAHA (25), for certain cytological processes in root tips; and the data given by WIESNER (24) for rate of germination of fungus spores indicate that he also encountered this phenomenon. The results of these studies cannot, however, be profitably compared with those of the present study.

It may be remarked that the separation of the individuals of a lot of seed into physiological categories, as such a separation or sorting results from studies like the ones here considered, is likely to be related to the general vitality of the seeds, to their varying degree of vital capacity in other respects as well as with regard to viability. It seems probable that the process of respiration may offer useful clues in this connection. If comparative studies on respiration might be conducted concomitantly with thoroughgoing experimentation on germinative energy enlightening results might be expected.

Summary and conclusion

This paper presents the results of experimental studies on the germinative energy of lots of seed, as the measure of this lot characteristic is influenced by the maintained temperature and by the length of the period of incubation employed in the experimental tests. Five lots of seed of pitch pine (*Pinus rigida* Mill.) were studied and several lots of seed of other coniferous trees. The seeds were usually subjected to a preliminary soaking in an antiseptic solution (to retard development of fungi), after which they were regularly distributed on the surfaces of agar plates, each in a glass Petri dish. The cultures thus prepared were themselves distributed among the incubation chambers for each experiment series. Thirty-six different maintained temperatures were employed, ranging from 8° to 57° C. and varying for the most part by intervals of one degree, and temperature fluctuation was usually not more than a single degree above or below the mean for the given chamber. Observations were made at daily intervals or more frequently, the length of the shortest period of incubation being 6–7 hours while that of the longest was 14–15 days. A seed was considered as having germinated when its radicle distinctly protruded from the split seedcoat. The number of seeds that had germinated in each culture was recorded for each incubation period and this number was expressed as a percentage of the total number of seeds in the culture, this percentage value being taken as a numerical index of germinative energy for that sample of seeds for that period and temperature. Mean index values were computed by averaging the corresponding percentage values for all similarly treated cultures. For each lot of seed a series of mean indices was thus obtained for each length of incubation period, there being a single mean index for each of the different maintained temperatures tested for that length of period.

The variables dealt with were: (1) the kind of seed tested (species and lot), (2) the maintained temperature and (3) the length of the incubation period. Temperature-germination graphs present the results, abscissas representing temperatures while ordinates represent the corresponding mean germinative-energy indices. A separate graph is presented for each length of incubation period tested, for each lot of seed studied.

The main aim of these studies was to ascertain approximately for each lot of seed the optimal maintained temperature for the occurrence of germination, for each length of incubation period tested. Some attention was given to the other cardinal temperatures also (minimal and maximal) and to the manner in which the form of the temperature-germination graph alters with the length of the incubation period.

For any germination to occur among the seeds of the lot of pitch pine seed to which this study was mainly devoted, the maintained-temperature maximum appears to have been above 57° , perhaps as high as 60° . The minimal maintained temperature is shown as about 44° for the shortest incubation periods (6 or 7 hours) that gave any germination at all. For progressively longer incubation periods the minimal maintained temperature appears to have been progressively lower and a 14-day period of incubation gave a minimum below 15° .

For the shortest incubation periods (6–7 hours) that gave any germination in this lot of pitch pine seed, the optimal maintained temperature was near the minimum, about 46° . For long periods (4–14 days) the optimal temperature appears as a single optimal range extending from about 23° to about 33° , the upper limit of the range being progressively lower for longer periods. For incubation periods of intermediate length a *double optimum* is clearly and unmistakably shown by the experimental data, a high-temperature optimum (corresponding to the single optimum for the shortest periods) and a low-temperature optimum (corresponding to the single optimum for the longest periods). For a 3-day period of incubation these two optima are represented by the temperature ranges from 27° to 33° and from 42° to 43° , respectively. The temperature-germination graph for this intermediate length of period consequently shows two high regions with a pronounced depression between. If a hypothetical graph for an incubation period of about 2.5 days is constructed by interpolation, the germinative-energy index for the high-temperature optimum (43° – 44°) becomes numerically the same (24 per cent.) as that for the low-temperature optimum (32° – 33°). This means that, for a properly chosen length of incubation period this lot of seed should be expected to exhibit about the same high percentage of germination with maintained temperatures about 33° and about 43° , while the expected corresponding value for an intermediate temperature about 36° – 37° is very much lower (about 7 per cent.).

The phenomenon of a double maintained-temperature optimum (two different optima) for the occurrence of germination in a lot of seed is here reported apparently for the first time. It is clearly shown for lot 1 of pitch pine seed and is indicated for lots 2 and 5, but was not evident for lots 3 and 4. It therefore appears to have characterized some, but not all, of the lots of pitch pine seed studied. Similar pairs of temperature optima are shown for single lots of seed of red pine (*Pinus resinosa* Ait.), lodgepole pine (*Pinus murrayana* Balf.), loblolly pine (*Pinus taeda* L.), longleaf pine (*Pinus palustris* Mill.) and Engelmann spruce (*Picea engelmanni* Engelm.), and they are suggested for Scotch pine (*Pinus sylvestris* L.) and smooth cypress (*Cupressus glabra* Sudw.). A single lot of rice seed (*Oryza*

sativa L.) was somewhat thoroughly studied in connection with the experimentation here reported, without the discovery of any indications of a double temperature optimum. It is possible that double optima may be found to characterize the temperature-germination relations of lots of many kinds of seed.

A lot of seed may be classified in groups or categories, according to the different proportions of its individuals that are capable of germination under the influence of different sets of temperature and duration conditions. For example, lot 1 of pitch pine seed, for which the most extensive data are available from this study, may be considered as having been made up of at least three different physiological forms or types: (A) seeds capable of germination in 14 days or less at a temperature about 25° but not at a temperature about 43°, (A') seeds capable of germination at both of these maintained temperatures within 14 days or less, and (B) the rest of the lot, not capable of germination in 14 days at any temperature, with the background conditions that were employed. These three categories appear to represent, respectively, about 65, 25 and 10 per cent. of the lot. Seeds of category A were permanently injured when incubated for a day or two at about 43° and they failed to germinate subsequently when transferred to a temperature about 25°. Seeds of category A' germinated very quickly at about 43° but they produced unhealthy seedlings, which grew but slowly even after being transferred to a temperature about 25°. The high-temperature optimum is thus seen to favor quick germination of the seeds of category A', but apparently at the expense of subsequent vitality in the seedlings produced.

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VITA

Ferdinand Wead Haasis was born January 25, 1889, in Brooklyn, N. Y., his parents being Dunbar Ferdinand Haasis and Hester Marsh Wead Haasis. His early education was received at home, in a small private school, and in the public schools of Perth Amboy and Rahway, N. J. In 1907 he entered Rutgers College, New Brunswick, N. J., and was graduated from that institution in 1911, receiving the degree of Bachelor of Science in Agriculture. In July, 1911, he entered the Forest School of Yale University, and was graduated with the degree of Master of Forestry in 1913. During summer vacations of 1910 and 1912 and from 1913 to 1915 he held various short-term positions with the Department of Botany of the New Jersey Agricultural Experiment Station, the New York State Conservation Commission, the United States Forest Service, the Connecticut Forestry Department, the North-Eastern Forestry Company, of Cheshire, Conn., and other organizations. From June, 1915, to August, 1917, he was technical assistant in the Forestry Department of the Consolidation Coal Company, at Jenkins, Ky. In August, 1917, he enlisted as a private in the Medical Department of the United States Army; and was discharged in February, 1919, as a battalion sergeant major of Infantry. On February 23, 1918, he was married to Bessie Ely Amerman, of New York. From March, 1919, to September, 1926, and during the summer of 1927, he was successively Forest Assistant, Forest Examiner, and Assistant Silviculturist at the Fort Valley Experiment Station (Flagstaff, Ariz.) and the Appalachian Forest Experiment Station (Asheville, N. C.) of the United States Forest Service. During the academic years 1926-27 and 1927-28 he was on furlough from the United States Forest Service and devoted this time to work in plant physiology at the Johns Hopkins University and to the work of a student assistant in the Department of Plant Physiology of that University.

